EFFECT OF HYDROALCOHOLIC EXTRACT OF *VITIS VINIFERA* LINN ON FEMALE WISTAR RATS WITH ESTRADIOL VALERATE INDUCED POLYCYSTIC OVARIAN SYNDROME

A Dissertation submitted to

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In partial fulfillment of the Requirement for the award of the degree of

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IN

BRANCH-IV -> PHARMACOLOGY

Submitted by

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Under the Guidance of

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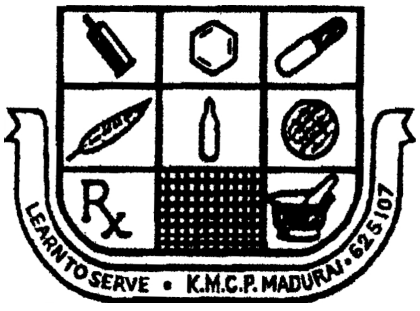

DEPARTMENT OF PHARMACOLOGY

K.M.COLLEGE OF PHARMACY

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CERTIFICATE

This is to certify that the dissertation entitled **"EFFECT OF HYDROALCOHOLIC EXTRACT OF *VITIS VINIFERA* Linn ON FEMALE WISTAR RATS WITH ESTRADIOL VALERATE INDUCED POLYCYSTIC OVARIAN SYNDROME"** submitted by **Mr. S. VINOTHKUMAR** in partial fulfillment for the degree of **"Master of Pharmacy** in **Pharmacology"** under The Tamilnadu Dr. M.G.R Medical University Chennai, at K.M.College of Pharmacy, Madurai–625107, is a bonafide work carried out by him under my guidance and supervision during the academic year of **2016 – 2017**. This dissertation partially or fully has not been submitted for any other degree or diploma of this university.

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This is to certify that the plant specimen brought to me by **Mr. S. VINOTHKUMAR,** Second year **M. Pharm (Pharmacology)**, Student of **K. M. College of Pharmacy, Madurai** has been identified as "***Vitis vinifera* Linn."** belonging to the family **"Vitaceae".**

Dr.D.Stephen.

Date ; 07/07/2016

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ACRONYMS

1. WHO World Health Organization
2. AES Androgen Excess and PCOS Society
3. AMH Anti-müllerian hormone
4. AR Androgen receptor
5. ASRM American Society for Reproductive Medicine
6. ATGL Adipose triglyceride lipase
7. CNS Central nervous system
8. CRF Corticotrophin-releasing factor
9. CRP C-reactive protein
10. CT Cycle threshold
11. CVD Cardiovascular disease
12. DHEA Dehydroepiandrosterone
13. DHEAS Dehydroepiandrosterone sulfate
14. DHT Dihydrotestosterone
15. EST Estrogen
16. EIA Enzyme immunoassay
17. ESHRE European Society for Human Reproduction and Embryology
18. Eg Example
19. FFA Free fatty acids
20. FSH Follicle stimulating hormone
21. GC-MS Gas chromatography-mass spectrometry
22. GDR Glucose disposal rate
23. GIR Glucose infusion rate
24. GnRH Gonadotropin-releasing hormone
25. HOMA Homeostasis model assessment
26. HPA Hypothalamic-pituitary-adrenal
27. HPO Hypothalamic-pituitary-ovarian
28. HSL Hormone-sensitive lipase
29. Hr hour
30. I.P Intraperitoneally
31. P.O orally

32. PCO Polycystic ovary
33. PCOS Polycystic ovary syndrome
34. POMC Pro-opiomelanocortin
35. PRGN Progestrone
36. QUICKI Quantitative insulin sensitivity check index
37. RIA Radioimmunoassay
38. SD Standard deviation
39. SEM Standard error of the mean
40. SHBG Sex hormone binding globulin
41. TSN Testosterone
42. VMC Vasomotor centre

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"The dream begins with a teacher who believes in you, who tugs and pushes you to the next plateau,sometimes poking you with a sharp stick called knowledge."

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CONTENTS

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine system disorder among women of reproductive age.[1]Metabolic derangements and associated complications include insulin resistance and diabetes, hyperlipidemia, hypertension, fatty liver, metabolic syndrome and sleep apnea.[2] Polycystic ovary syndrome (PCOS) is a common condition characterized by menstrual abnormalities and clinical or biochemical features of hyperandrogenism, altered LH: FSH ratio (>2/3:1).[3] Features of PCOS may manifest at any age, ranging from childhood (premature puberty), teenage years (hirsutism, menstrual abnormalities), early adulthood and middle life (infertility, glucose intolerance) to later life (diabetes mellitus and cardiovascular disease.[4-6]

PCOS is now recognised to be a metabolic syndrome which may include hyperinsulinaemia, hyperlipidaemia, diabetes mellitus, and possibly cardiac disease, as well as the more conventionally recognised increase in androgen levels, cosmetic problems, anovulation, infertility, endometrial cancer and obesity.[7,8] PCOS is estimated to affect between 5 and 10 percent of women of reproductive age, affects women of all races and nationalities. Women with PCOS may have enlarged ovaries that contain small collections of fluid called follicles located in each ovary. Polycystic means the ovaries have many cysts or follicles that rarely grow to maturity or produce eggs capable of being fertilized.The presence of polycystic ovaries on ultrasound examination is particularly controversial as a criterion. Polycystic ovaries are characterised by peripheral cysts (10 or more) less than 10mm in size in an enlarged ovary with significant increase in the central stroma.[9]

PCOS is a set of symptoms that result from a hormonal imbalance affecting women and girls of childbearing age. Infrequent or prolonged menstrual periods, excess hair growth, acne, and obesity can all occur in women with polycystic ovary syndrome. A condition in which women have high levels of male hormones, increasing the risk of irregular or absent menstrual cycles, infertility, obesity, Women with PCOS usually have at least two of the following three conditions:

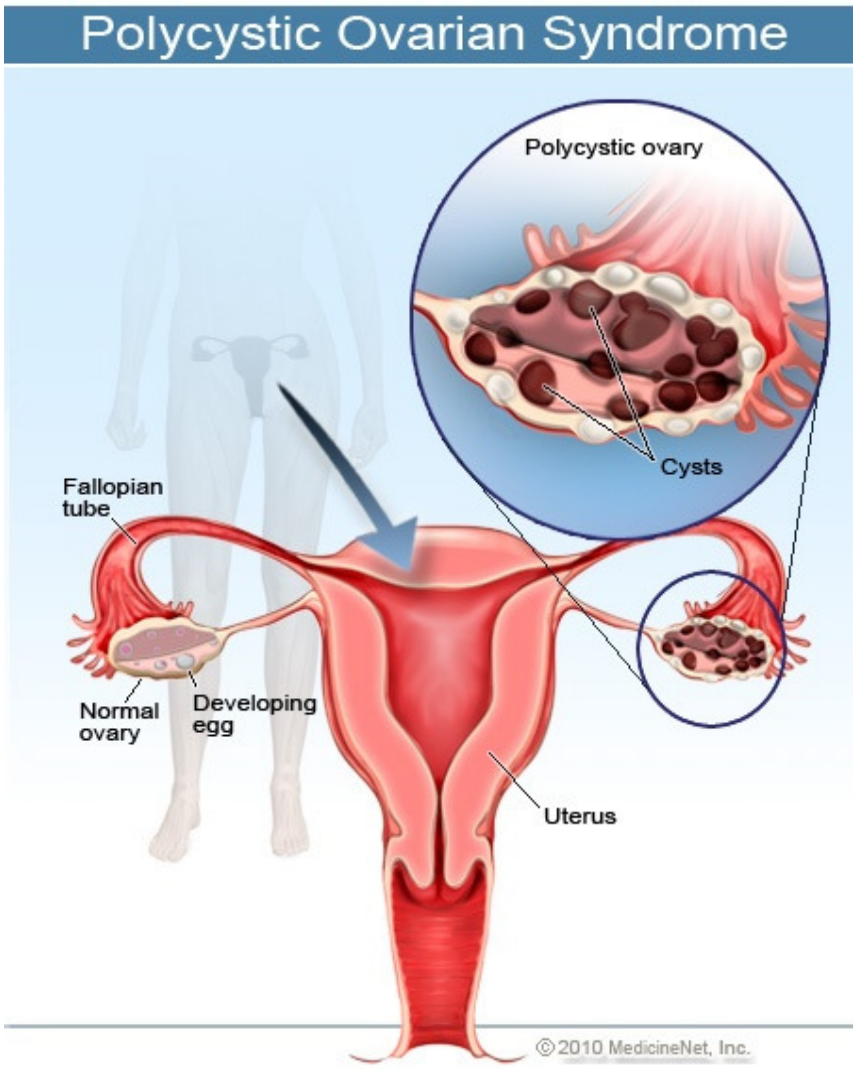


Fig.1: Poly Cystic Ovary Syndrome

- Absence of ovulation, leading to irregular menstrual periods or no periods at all
- High levels of androgens (a type of hormone) or signs of high androgens, such as having excess body or facial hair
- Cysts (fluid-filled sacs) on one or both ovaries—"polycystic" literally means "having many cysts.

In addition, women with PCOS display reduced health-related quality of life as well as symptoms of anxiety and depression [10,11].

Epidemiology

Several studies have suggested a prevalence of PCOS of 5%– 10% in women of reproductive age, using the diagnostic criteria of the US National Institutes of Health.[12,13] Polycystic ovaries alone were found in 20%–25% of women in surveys in the United Kingdom and New Zealand.[14,15] While women with polycystic ovaries and no evidence of menstrual dysfunction or hyperandrogenism appear normal, they do have an overexaggerated response to stimulation with gonadotrophins such as follicle-stimulating hormone (FSH) in cycles of assisted reproduction. PCOS is generally underdiagnosed, and clinicians should remember that menstrual abnormalities, such as cycles shorter than 21 days or longer than 35 days, are often associated with the condition. Many young women with these abnormalities are prescribed the oral contraceptive pill, which masks the condition until they try to achieve pregnancy.[16]

Pathogenesis

The pathogenesis of PCOS is poorly understood, but the primary defect may be insulin resistance leading to hyperinsulinaemia. In the ovary, the cardinal feature is functional hyperandrogenism. Circulating concentrations of insulin and luteinising hormone (LH) are generally raised. The theca cells, which envelop the follicle and produce androgens for conversion in the ovary to oestrogen, are over responsive to this stimulation. They increase in size and overproduce androgens. The rise in LH levels is thought to be caused by the relatively high and unchanging concentrations of oestrogens that may alter the control of this hormone by the hypothalamic–pituitary axis. This combination of raised levels of androgens, oestrogen, insulin and LH explains the classic PCOS presentation of hirsutism, anovulation or dysfunctional bleeding, and dysfunction of glucose metabolism. Paradoxically, although the insulin regulatory molecules on the theca cells are responsive to insulin those in the muscle and liver are resistant.[17] Dysregulation of cytochrome p450c17, the androgen-forming enzyme in both the adrenals and the ovaries, may be the central pathogenic mechanism underlying hyperandrogenism in PCOS. In the presence of 5-alpha-reductase, testosterone is converted within the cell to the more potent androgen

dihydrotestosterone. Excess 5-alphareductase activity in the skin determines the presence or absence of hirsutism.[18]

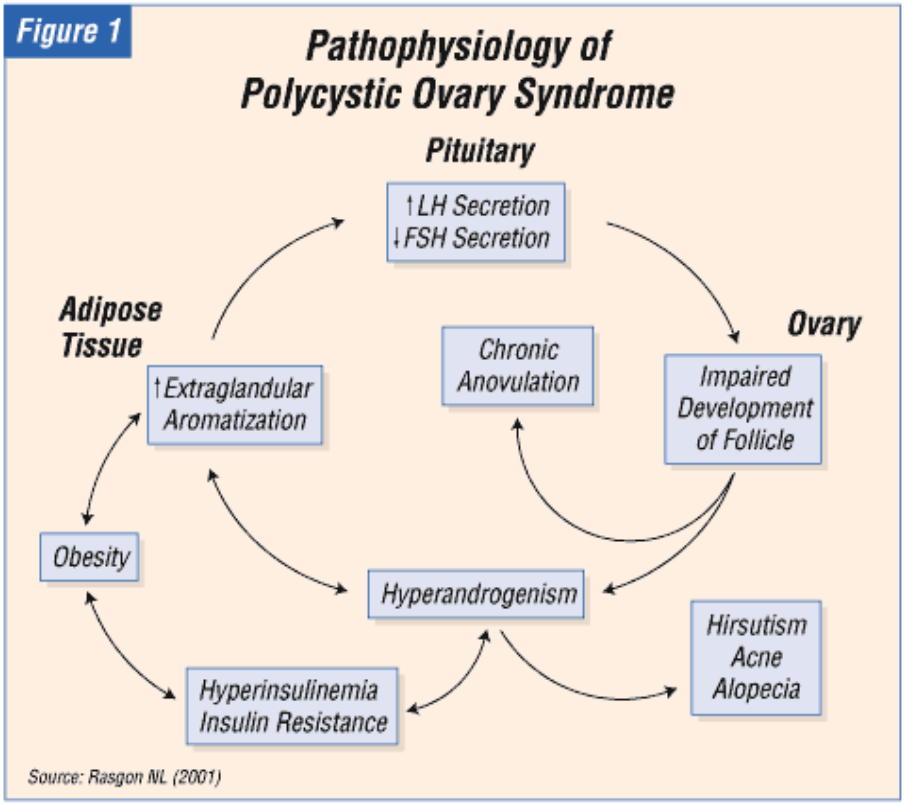


Fig 2.Pathophysiology of polycystic ovary syndrome

Etiology of PCOS

PCOS is a hormonal condition commonly involving high levels of insulin or male hormones known as 'androgens', or both. PCOS is caused by an imbalance in the hormones (chemical messengers) in brain and ovaries. PCOS develops when the ovaries overproduce androgens (e.g., testosterone). Androgen overproduction often results from overproduction of LH (luteinizing hormone) by pituitary gland which results in extra testosterone production by the ovary.

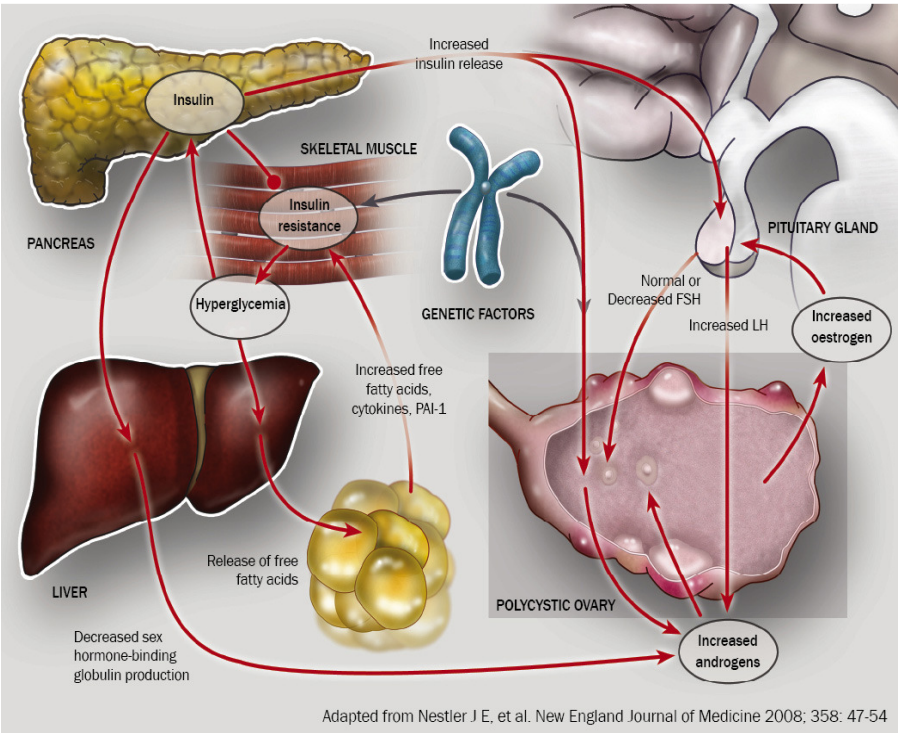


Fig.3 Etiology of Poly Cystic Ovary Syndrome

1. The pituitary gland in your brain makes the hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH).

2. After getting the signal from the hormones LH and FSH, the ovaries make estrogen and progesterone the female sex hormones.

3. All normal ovaries also make a little bit of the androgen testosterone a male sex hormone. The pancreas is an organ that makes insulin. High levels of insulin can also cause the ovaries to make more of the hormone testosterone.

The exact cause of polycystic ovary syndrome (PCOS) is unknown, but it's thought to be related to abnormal hormone levels.

Resistance to insulin

Insulin is a hormone produced by the pancreas to control the amount of sugar in the blood. It helps move glucose from blood into cells, where it is broken down to produce energy.

Insulin resistance means the body's tissues are resistant to the effects of insulin. The body therefore has to produce extra insulin to compensate.

High levels of insulin cause the ovaries to produce too much testosterone hormone, which interferes with the development of the follicles (the sacs in the ovaries where eggs develop) and prevents normal ovulation.

Insulin resistance can also lead to weight gain, which can make PCOS symptoms worse because having excess fat causes the body to produce even more insulin.[19]

Hormone imbalance

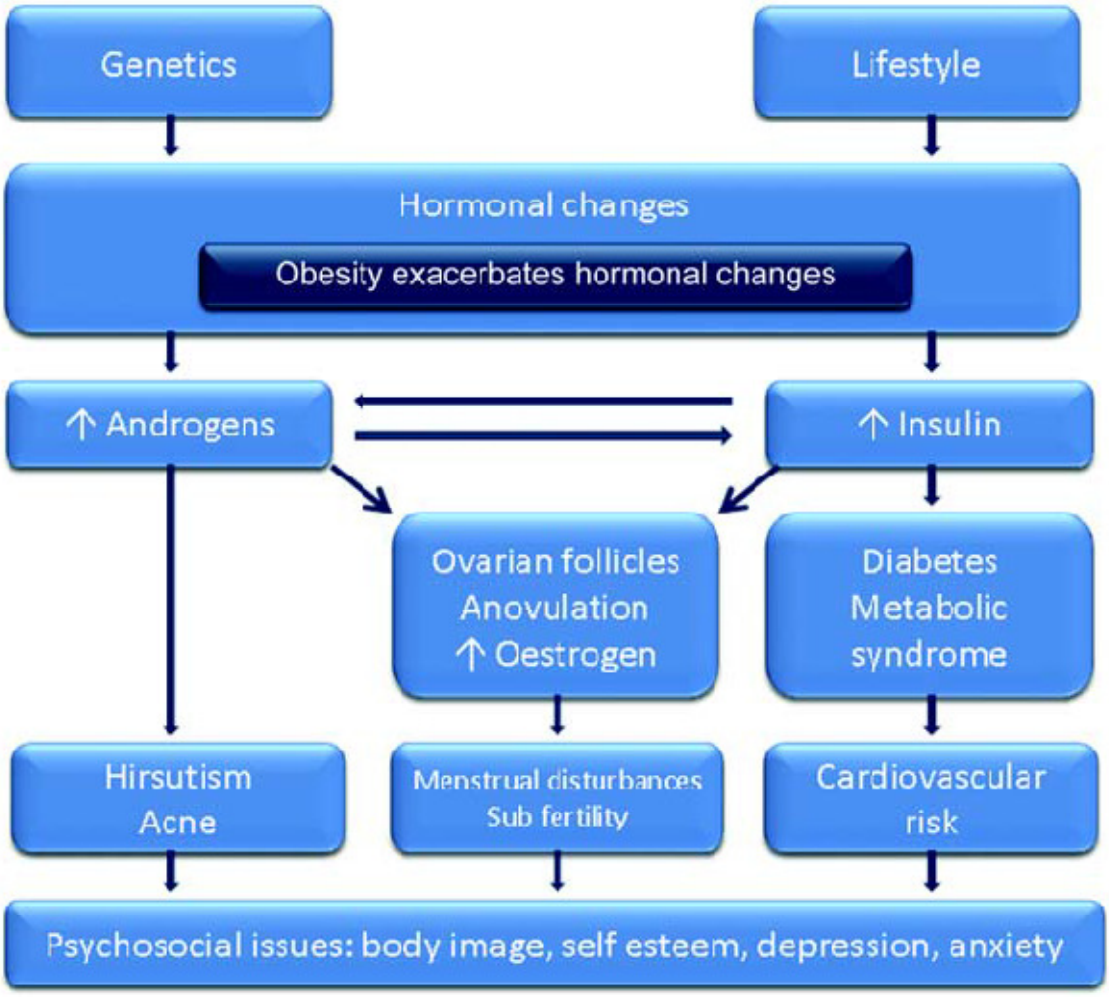


Fig. 4. Hormonal imbalance during PCOS

Many women with PCOS are found to have an imbalance in certain hormones, including:

➲ **Raised levels of testosterone** - a hormone often thought of as a male hormone, although all women normally produce small amount of it.

➲ **Raised levels of luteinizing hormone (LH)** – a hormone that stimulates ovulation, but may have an abnormal effect on the ovaries if levels are too high.

➲ **Low levels of sex hormone binding globulin (SHBG)** – a hormone that helps reduce the effect of testosterone.

➲ **Raised levels of prolactin (only in some women with PCOS)** – a hormone that stimulates the breast glands to produce milk in pregnancy.

The exact reason why these hormonal changes occur is not known. It's been suggested that the problem may start in the ovary itself, in other glands that produce these hormones, or part of the brain that controls their production. The changes may also be caused by the resistance to insulin.[20]

Causes of PCOS

Following are few important causes [21] of PCOS:

1) Genetic predisposition

2) Strong stimulation in adrenals in childhood

3) Raised insulin levels

4) Contraceptive pills

5) Hormonal imbalance

6) Stress

PCOS and age

More and more evidences indicate that PCOS appears to be a pathology that involves "the whole" life of the woman, that begins in the intra uterine life in genetically predisposed individuals, occurs at the time of puberty, endures in child

bearing age and exposes, especially after menopause, to a higher risk of developing endometrial cancer, cardiovascular disease, hypertension, diabetes mellitus type 2. For these reasons it is clear that a correct and early diagnosis of this syndrome is fundamental, as it allows to perform the most appropriate treatments and check-ups thus reducing the risk of developing all the complications related to it.

Symptoms of polycystic ovarian syndrome:

Women who have PCOS [22] may experience:

- irregular menstrual cycles – menstruation may be less or more frequent due to less frequent ovulation (production of an egg)
- amenorrhoea (no periods) – some women with PCOS do not menstruate, in some cases for many years
- Hirsutism (excessive hair growth on the face, chest, abdomen, etc.) possibly due to increased free testosterone
- Hair loss (androgenic alopecia, in a classic "male baldness" pattern)
- Acne
- Polycystic ovaries
- Obesity
- Infertility or reduced fertility
- mood changes – including anxiety and depression

In addition, women with PCOS appear to be at increased risk of developing the following health problems during their lives:

- Insulin resistance
- Diabetes
- Lipid abnormalities
- Obstructive sleep apnea
- Cardiovascular disease
- Endometrial carcinoma (cancer)

Manifestations of PCOS

PCOS is a lifelong condition which may have effects at all ages, not just in the reproductive years.

Fetal life

The condition may have its origins in fetal life, with either intrauterine growth retardation or post-term birth. Researchers have claimed that these children are more prone to hyperinsulinism, premature pubarche and signs of PCOS early in reproductive life.[23,24]

Teenagers will often have oligo- or amenorrhoea, hirsutism, acne and weight disorders. It is controversial whether patients with PCOS suffer from a raised prevalence of bulimia.

Women seeking to become pregnant will have difficulties because of anovulation and later may be concerned about overweight and hirsutism. It is controversial whether miscarriage is increased in PCOS, or whether pregnancy loss is a result of excess body weight.

Diagnosis of PCOS

The diagnostic criteria for PCOS are controversial but diagnosis is generally based on peripubertal onset of menstrual problems with clinical or biochemical hyperandrogenism. Polycystic ovaries are characterised by peripheral cysts (10 or more) less than 10mm in size in an enlarged ovary with significant increase in the central stroma.[25] Polycystic ovaries are also found in women with no evidence of menstrual dysfunction or hyperandrogenism.[26,27]

Diagnosis of PCOS is now largely based on the Rotterdam criteria which are inclusive of the original National Institutes of Health (NIH) criteria15 and require two of three key features: oligo- or anovulation, clinical and/or biochemical hyper androgenism and polycystic ovaries . However, as noted, PCOS phenotypes vary widely depending on life stage, genotype, ethnicity and environmental factors, including lifestyle and body weight.

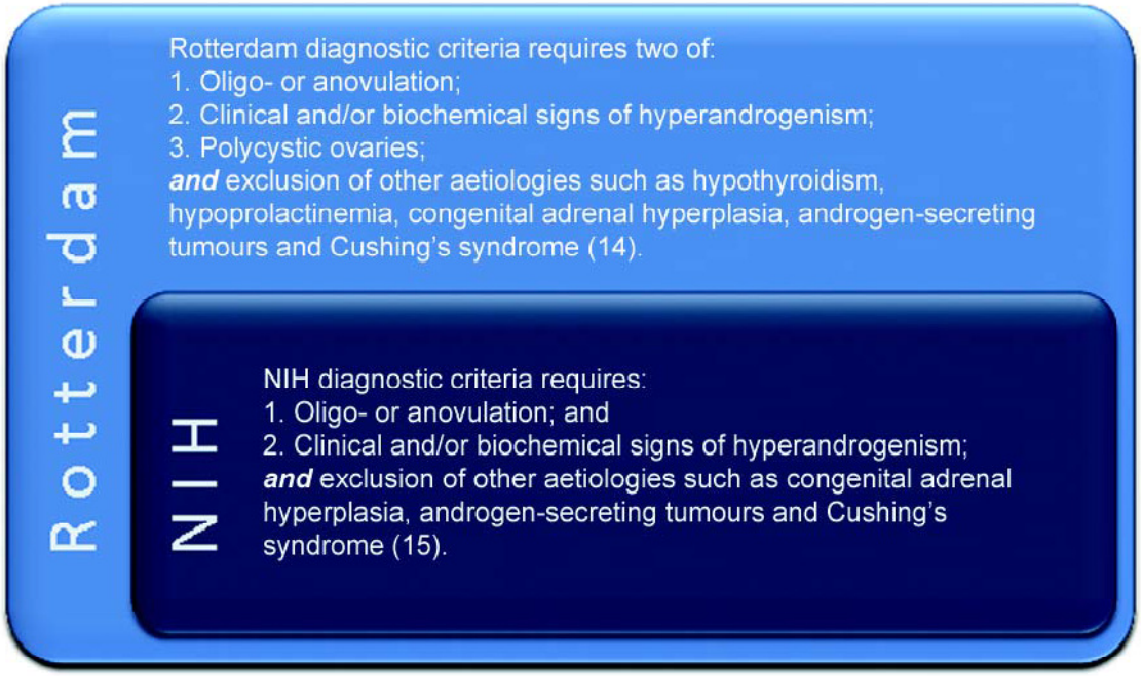


Fig.5. Diagnosis of PCOS

Diagnosis of PCOS is likely to involve:

- Medical history
- Ultrasound, to assess whether ovaries are enlarged and cystic.
- Blood tests, to detect elevated levels of androgens.
- Blood test to detect high levels of LH (luteinizing hormone) or an elevation in the ratio of LH to FSH (follicle stimulating hormone).
- Monitoring of the ovary's response to either a stimulatory dose of gonadotropin-releasing hormone agonist

Early diagnosis is important as it can allow symptoms to be managed and may prevent the development of long-term health problems such as diabetes.

Diagnostic investigations must exclude other causes and include thyroid function tests and prolactin and follicle stimulating hormone (FSH) levels.[28]

PCOS AND THE METABOLIC SYNDROME

Features of the metabolic syndrome, including obesity, insulin resistance and dyslipidaemia, are common in women with PCOS.

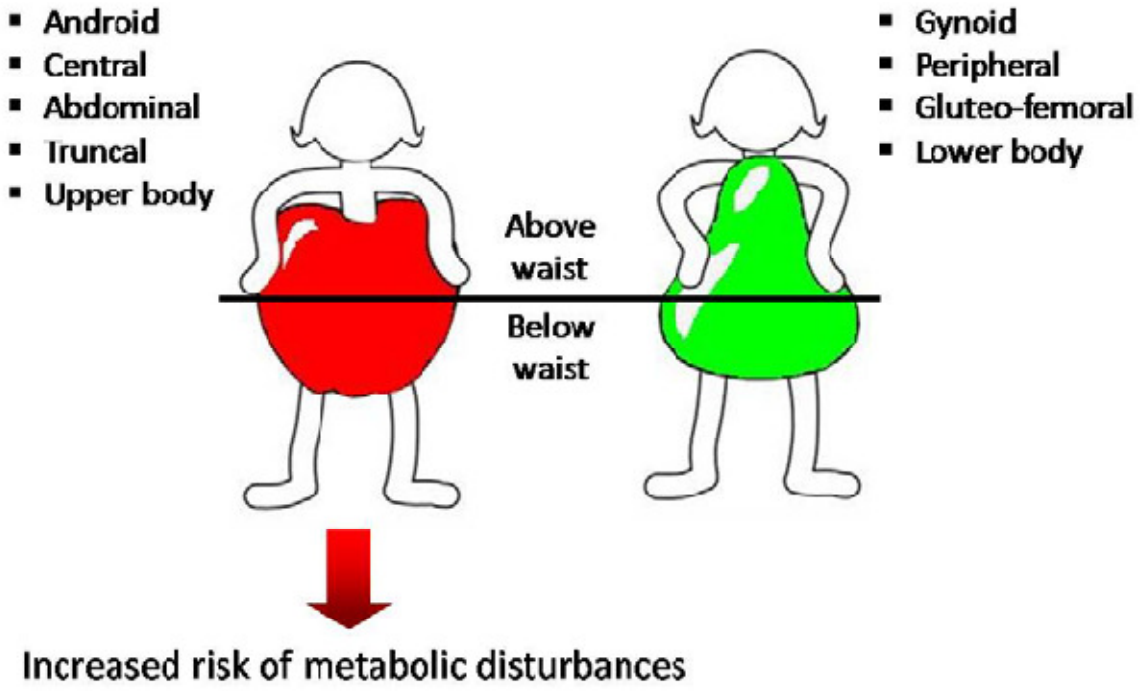


Fig.6. PCOS and the metabolic syndrome

OBESITY:

The incidence of obesity in women with PCOS varies between countries and ethnic groups. In the United States, about 50% of women with PCOS are overweight or obese, but this prevalence differs little from that in the, general community. In other countries, PCOS appears to be associated with obesity, but at a lower rate than in the US. Obesity tends to be central (abdominal) in its distribution, and even lean women with PCOS may have a fat distribution favouring central omentaland visceral fat.

INSULIN RESISTANCE:

This is independently related to PCOS, with women of normal weight with PCOS showing a degree of hyperinsulinaemia and impaired glucose disposal after meals and during glucose tolerance tests (oral or intravenous).[29] It is uncertain whether this insulin resistance results from a specific genetic post-receptor defect, such as a defect in serine phosphorylation,[30] or whether it is comparable to the problem seen in type 2 diabetes. Certainly, hyperinsulinism is common but is difficult to interpret clinically, given the fact that it also results from obesity.

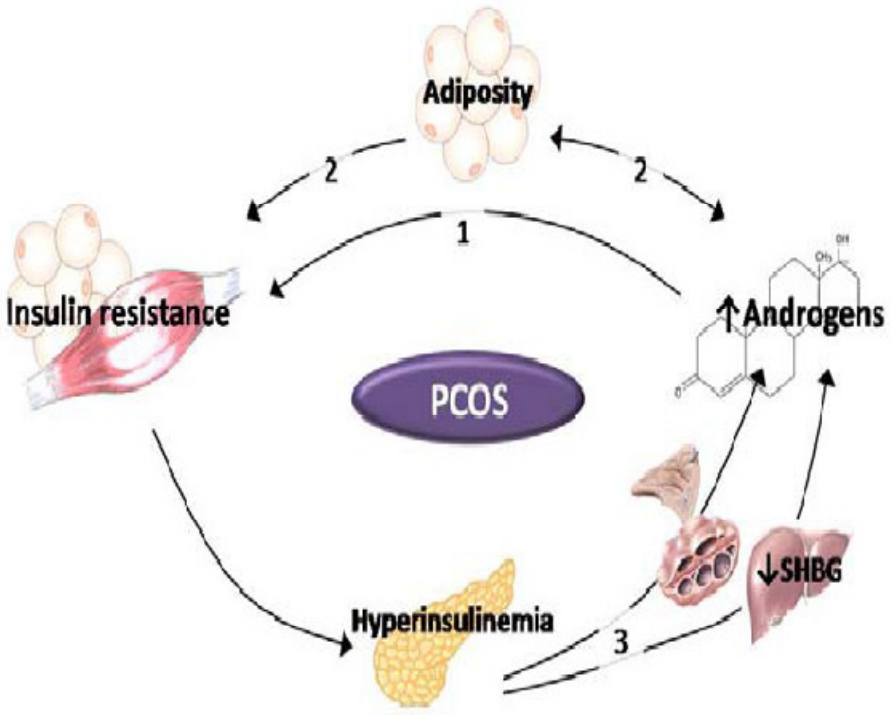


Fig.7.Insulin resistance and PCOS

IMPAIRED GLUCOSE TOLERANCE AND TYPE 2 DIABETES:

`These are major complications in overweight women with PCOS. While fasting glucose level is usually normal, insulin release after a glucose load is increased, and glucose disposal is impaired. An excellent epidemiological study in the UK that followed up women with a histological diagnosis of PCOS after wedge resection of the ovaries found clear evidence of an increase in the rate of diabetes.[31] This confirmed the results of many other studies from the US and Europe. In obese

women with PCOS, progression from normal glucose function to impaired glucose tolerance or diabetes mellitus is more rapid than in women without PCOS.[32]

DYSLIPIDAEMIA:

Hypertriglyceridaemia, increased concentrations of low-density lipoprotein (LDL) cholesterol and decreased concentrations of high-density lipoprotein (HDL) cholesterol are common in women with PCOS, particularly if obese. Levels of plasminogen activator inhibitor-1 may also be raised, suggesting a chronic underlying inflammatory- like process

CARDIOVASCULAR DISEASE:

The metabolic features of PCOS have led to widespread concern about the risk of cardiovascular disease. A higher than expected prevalence of PCOS has been reported among young women with angiographically proven narrowing of the coronary vessels; women with PCOS were also more likely to have sonographic evidence of premature obstruction of other large vessels.[33,34] However, a UK study of medical records and death certificates of women with a histological diagnosis of PCOS revealed no evidence for an increase in myocardial infarction or other types of heart disease.

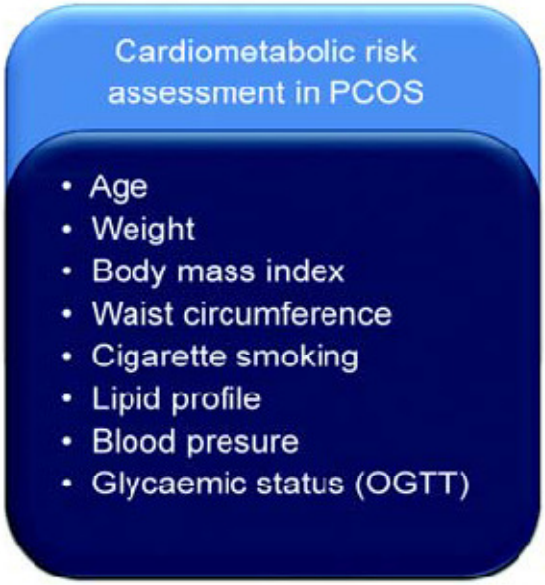
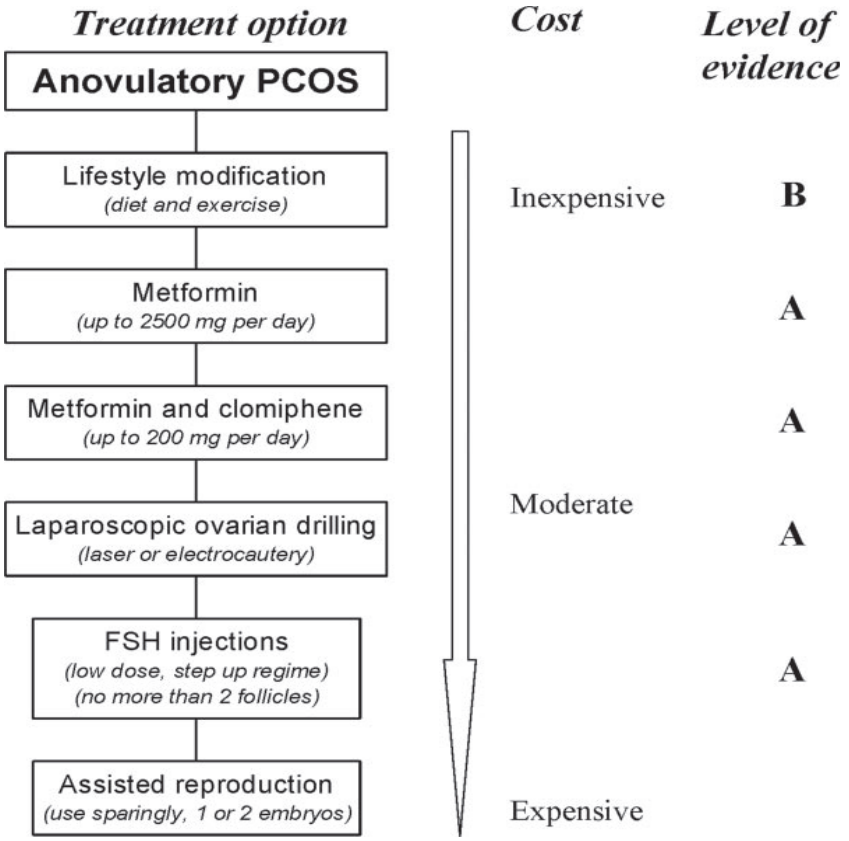


Fig.8.Cardiometabolic risk assessment in PCOS

TREATMENTS AND DRUGS

Polycystic ovary syndrome treatment generally focuses on management on main concerns, such as infertility, hirsutism, acne or obesity. Regular exercise, a diet, weight control, and not smoking are all important parts of treatment for polycystic ovary syndrome (PCOS) There is no cure for PCOS, but controlling it lowers your risks of infertility, miscarriages, diabetes, heart disease, and uterine cancer.



MANAGEMENT

Management comprises treatment of the presenting symptoms, as well as any other abnormalities discovered on investigation. Depending on the problems, management of PCOS can include lifestyle modifications, weight reduction, and treatment with hormones or medications. The modality depends on the desire for fertility Research has shown that even a five to 10 per cent loss of weight in those

who are overweight can restore normal hormone production and helps regulate periods and improve fertility.

Hirsutism

Hirsutismshould be assessed qualitatively or semiquantified using the FerrimanGallwey score.[35]

Treatment may include:

- The oral contraceptive pill (eg, ethinyloestradiol 35 μg plus cyproterone acetate 2mg daily for 21 of 28 days);
- Cosmetic measures (eg, laser electrolysis, bleaching, waxing or shaving)
- Oraloestrogen and cyproterone acetate (oestradiolvalerate 2mg daily and cyproterone acetate 50 mg for 14 days a month);
- Spironolactone (75–200mg daily); or
- Other drugs, such as the antiandrogenflutamide or the antifungal agent ketoconazole. These drugs either reduce androgen production or inhibit androgen-binding to the receptor. They are not in general use for this purpose in Australia. Response times for drugs can be up to 3 months.

Fig. 9. Women with hirsutism

MENSTRUAL DYSFUNCTION AND ENDOMETRIAL HYPERPLASIA

Menstrual Dysfunction, including irregular periods, can be managed by administration of progestins (eg, medroxyprogesterone acetate or norethisterone) or the oral contraceptive pill.

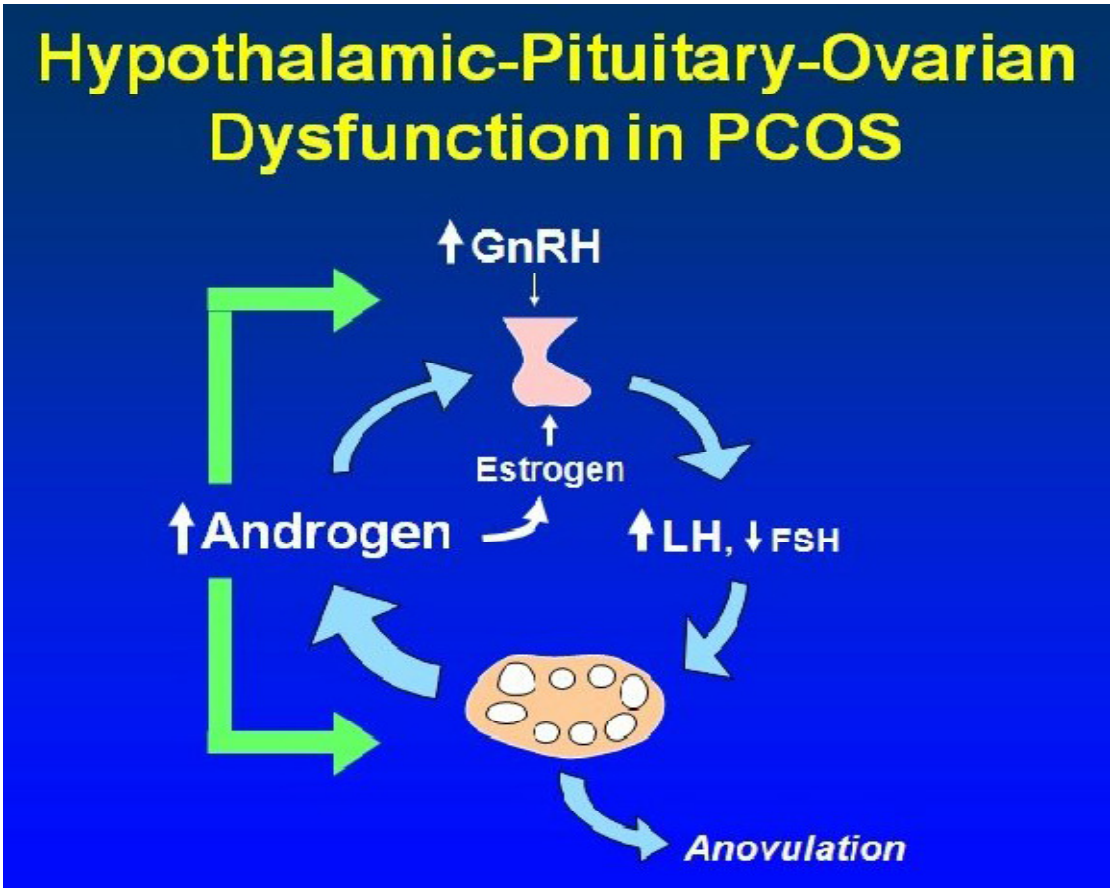


Fig.10.Hypothalamic-Pituitary-Ovarian Dysfunction in PCOS

Endometrial Hyperplasia should be assessed by ultrasound examination, endometrial biopsy or hysteroscopy, and can be treated by hormonal therapy, such as the oral contraceptive pill or progestins.

Comparison of Regular Menstrual Cycle with PCOS Cycle

1.The menstrual cycle starts when the brain sends LH and FSH to the ovaries. A big surge of LH is the signal that causes the ovaries to ovulate, or release an egg.

2.The egg travels down the fallopian tube and into the uterus. Progesterone from the ovary causes the lining of the uterus to thicken.

3.If the egg isn't fertilized, the lining of the uterus is shed. This is a menstrual period.

4. After the menstrual period, the cycle begins all over again.

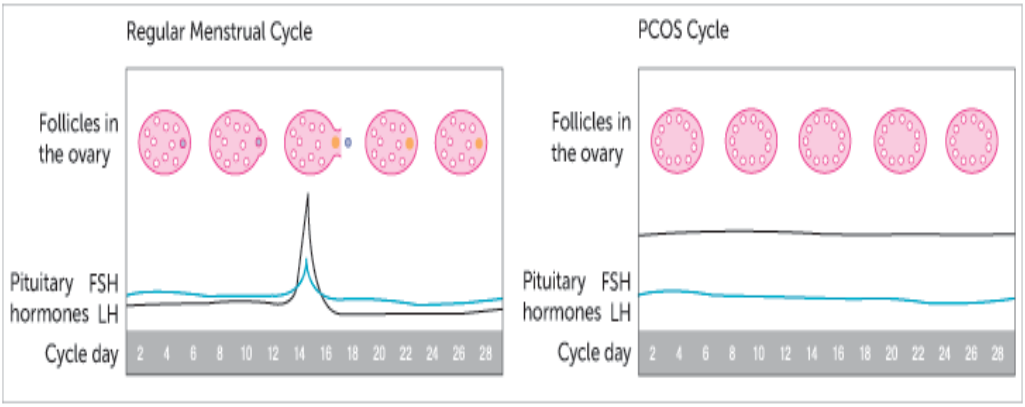


Fig.11.Comparition of Normal and PCOS Menstrual Cycle

The most common form of treatment for PCOS is the birth control pill; however, other kinds of hormonal therapy may include the "vaginal ring" and "the patch". Even if you're not sexually active, birth control pills may be prescribed because they contain the hormones that your body needs to treat your PCOS. By taking the birth control pill either continuously or in cycles you can:

- Correct the hormone imbalance
- Lower the level of testosterone (which will improve acne and lessen hair growth)
- Regulate your menstrual periods
- Lower the risk of endometrial cancer (which is slightly higher in young women who don't ovulate regularly)
- Prevent an unplanned pregnancy if you are sexually active

OVERWEIGHT, OBESITY AND GLUCOSE INTOLERANCE

Lifestyle changes are a first-line intervention in women with PCOS who are overweight.[36] Glucose intolerance can be managed by diet and exercise, weight control and oral antidiabetic drugs (eg, metformin).

INFERTILITY

The cause of infertility in patients with PCOS is generally lack of ovulation because of a failure of the follicles to develop beyond 10mm. Most cycles are anovulatory, and induction of ovulation is essential.

LIFESTYLE MODIFICATION:

Several studies have shown that weight loss can lead to resumption of ovulation within weeks. Clark and colleagues demonstrated that even a 5% reduction in body mass restores ovulation and fertility[37,38] and devised a program of exercise and sensible eating that has become a model across the world for treating PCOS. Rapid changes in body composition and fat mass can be shown during lifestyle change. High-protein diets seem to be as effective as high-carbohydrate diets, provided that fat and total calories are comparable. while lifestyle changes are difficult to maintain, women seeking pregnancy are highly motivated, making this a first-line intervention in overweight women with PCOS. Longer-term changes in weight are more difficult to maintain.

CLOMIPHENE CITRATE:

Clomiphene (Clomid, Serophene) is an oral anti-estrogen, may add metformin to help induce ovulation. This is an oral oestrogen antagonist that raises circulating concentrations of FSH and induces follicular growth in most women with PCOS and anovulation. The initial regimen is 25–50mg per day for 5 days. Therapy can be monitored by oestrogen levels, follicular ultrasound examination and luteal progesterone level (>20nmol/L). Failure of response is associated with high body mass index and high androgen levels. Doses up to 200mg per day can be used before

failure of response is established. In the rare situation in which side effects limit treatment, Tamoxifen can be used. Both treatments increase the risk of multiple pregnancy.

LETROZOLE:

Another medication letrozole works to stimulate the ovaries, but it may help with ovulation when other medications fail.

METFORMIN:.

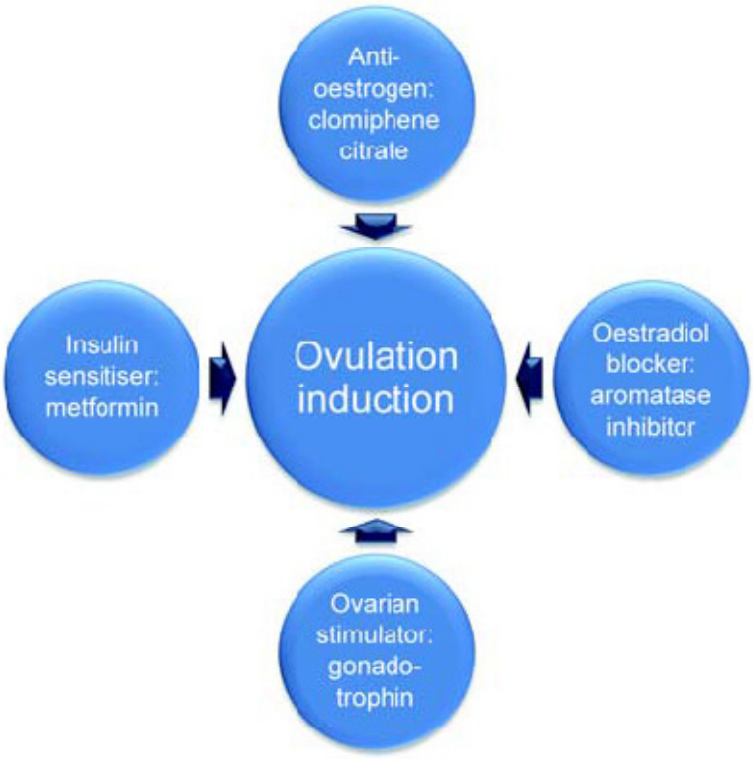
Prescribing Metformin (Glucophage, Fortamet, others), an oral medication for type 2 diabetes that improves insulin resistance and lowers insulin levels. This drug may help with ovulation and lead to regular menstrual cycles. Metformin also slows the progression to type 2 diabetes and aids in weight loss .Use of the insulin-sensitizing drug Metformin at doses of 500–2500mg daily is controversial, but appears valuable in increasing menstrual cyclicity and pregnancy rate. A recent consensus statement from the Endocrine Society of Australia indicated its use in women trying to become pregnant.[39-42] The new insulin-sensitizing agents, the "glitazones" — troglitazone (now discontinued), rosiglitazone and pioglitazone — have been shown to be very effective for ovulationinduction,[43]but are not approved by the Pharamaceutical Benefits Scheme for PCOS. There is greater concern about the effects on the fetus of these drugs compared with Metformin, and they should not be used by women trying tobecome pregnant.[44]

Another medication letrozole works to stimulate the ovaries, but it may help with ovulation when other medications fail.

SURGERY TO THE OVARIES:

Wedge resection of the ovaries has been abandoned because of concerns about pelvic adhesions, another cause of subfertility, and loss of valuable ovarian tissue. Ovarian diathermy or laser drilling has been used in recent years with apparently good results; a recent systematic review comparing drilling with clomiphene citrate and gonadotrophins proved equivalence in the studies examined.[45]However, like wedge resection, this surgery may produce pelvic adhesions. Destructive surgery to the ovary

should be used only after extensive discussion with the patient and not because the ovaries are found to be polycystic incidentally during routine laparoscopy.



GONADOTROPHIN TREATMENT:

Ovulation induction with gonadotrophins such as FSH has proved successful for at least three decades, but demands skill and experience to avoid multiple pregnancies and ovarian hyperstimulation syndrome. Patients start on low-dose recombinant FSH administered subcutaneously. Monitoring of ovarian response involves ultrasound examination, often with oestradiol measurement. Human chorionic gonadotrophin is given when one follicle reaches 16–20mm in size. Any more than two follicles of an appropriate size give the risk of multiple pregnancies. Multiple gonadotrophin cycles may be required to achieve pregnancy, but this approach is preferable before more invasive procedures, such as in-vitro fertilisation.

IN-VITRO FERTILISATION:

Provided there is no problem other than anovulation, this has little place in the management of infertility resulting from PCOS. Ovulation induction by a skilled reproductive endocrinologist is preferable to in-vitro fertilisation because of the risks of hyperstimulation and multiple pregnancies with the latter procedure.

LONG-TERM MANAGEMENT:

Women with PCOS require ongoing surveillance to detect impaired glucose tolerance, hyperlipidaemia, endometrial hyperplasia and consequent complications. Obese women, in particular, require regular (possibly annual) glucose tolerance testing because of the potential for rapid progression from normal to impaired glucose tolerance and diabetes.[46] Some investigators have suggested prophylactic use of Metformin in young teenagers and older women to avoid the problems of the metabolic syndrome. This approach is probably premature at present and is not recommended. Advice about improved exercise and diet is more rational, given the abundant data on the role of lifestyle change in preventing and treating problems of glucose metabolism.

REGULATE YOUR MENSTRUAL CYCLE:

To regulate menstrual cycle, recommended combination birth control pills — pills that contain both estrogen and progestin. These birth control pills decrease androgen production and break the effects of continuous estrogen, lowering risk of endometrial cancer and correcting abnormal bleeding. An alternative approach is to take progesterone for 10 to 14 days every one to two months. This type of progesterone therapy regulates the periods and offers protection against endometrial cancer.

REDUCE EXCESSIVE HAIR GROWTH:

Spironolactone (Aldactone) that blocks the effects of androgens on the skin. Because spironolactone can cause birth defects, effective contraception is required when using the drug, Eflornithine (Vaniqa) is another medication possibility; the cream slows facial hair growth in women.

HORMONE THERAPY:

To correct menstrual cycle problems, birth control hormones keep endometrial lining from building up for too long. This can prevent uterine cancer. Hormone therapy also can help with male-type hair growth and acne. Birth control pills, patches, or vaginal rings are prescribed for hormone therapy. Androgen-lowering spironolactone (Aldactone) is often used with combined hormonal birth control. This helps with hair loss, acne, and male-pattern hair growth on the face and body (hirsutism).

REGULAR CHECKUPS:

Regular checkups are important for catching any PCOS complications, such as high blood pressure, high cholesterol, uterine cancer, heart disease, and diabetes.[47-50]

ANIMAL MODELS FOR THE STUDY OF PCOS

To identify the complex nature of this disease, suitable animal models are needed. Researchers during the past three decades have identified different animal models that mimic many of the features of PCOS in women. These models have afforded valuable information into complex nature of PCOS. Currently, although a genetic component to PCOS has been identified, a specific "PCOS gene" has not, and therefore a specifically targeted gene deletion for PCOS as an animal model is not available. Therefore, the majority of PCOS models that are available to date rely upon external chemical treatments with steroids, steroid precursors or steroid receptor antagonist to achieve the pathology. Many of these models do not produce consistent results or cease to produce PCOS like symptoms once the treatment stops.[51] noted that their model for cardiovascular disease, the hypothalamic pro-opiomelanocortin (POMC) specific leptin-insulin double receptor knockout mouse exhibited some similar features to PCOS. Our recently developed theca specific Esr1specific knockout mouse model reproduces the clinical pathologic features of PCOS in 100% of animals.

Androgens

Within the ovary, androgens, mainly androstenedione (A4) and testosterone (T), are synthesized in the theca cells. A direct role for androgen receptor (AR)-mediated effects in the ovary and female reproductive functions has been recently confirmed by findings from AR knockout mouse models, where a loss of AR actions lead to subfertility, predominantly due to defective gonadotropin regulation, follicular development, and ovulation.[52, 53]

Prenatal Androgen

Prenatal androgen (PNA) treatment in sheep and monkeys results in multiple metabolic and reproductive abnormalities.

Pre-Pubertal Androgen

This model exploits the association of elevated androgen levels during puberty and PCOS. Administration of exogenous androgens results in permanent damage to the ovarian tissue and recapitulated the hallmark symptoms of PCOS in an animal model. PPA model shows many similar features to PCOS in women with the exception of the hallmark increase in basal LH levels. [54,55]

Letrozole

Letrozole is an oral non-steroidal aromatase inhibitor. Inhibition of aromatase prevents the conversion of androgens to estrogens and therefore this model has similar features to the prepubertal androgen treatment.[56]Similarly to pre-pubertal androgen, this model is also reliant on artificial hyperandrogenemia and does not help identify abnormalities upstream of hyperandrogenemia.

Dehydroepiandrosterone (DHEA)

DHEA is sufficient to induce a hyperandrogenizedstate in PCOS . This model is also reliant on artificial hyperandrogenemia and does not help identify abnormalities upstream of hyperandrogenemia.

RU486

Administration of RU486 on the day of estrus in rats exhibit increased basal LH, polycystic ovaries, ovulation blockade and metabolic defects.[57] However, this model is reversible and symptoms decrease upon cessation of the antiprogestin treatment.

Estradiol

The immune system is now a well-recognized component of reproductive biology. Immune cells are involved in all aspects of normal reproductive function including ovulation, corpus luteal formation, uterine receptiveness and maintenance of pregnancy. Chapman and collea-gues[58] now provides evidence that aberrant immune function may play a role in the pathogenesis of PCOS and that PCOS may result from as autoimmune disease. As a consequence of this, increased vascular permeability in the thymus allows auto reactive T-cells to escape into the circulation. The ultimate effect of these "escapees" is damage to tissues throughout the body including the ovaries. This model offers an abrupt change in the direction of PCOS research. The major deficiencies in this model are a lack of hyperandrogenemia and the fact that the loss of regulatory T-cell function would not only impact the ovary, but multiple other tissues resulting in pathologies not associated with PCOS. It will be interesting to follow the progress of this novel concept of autoimmune responses resulting in PCOS in women and if, PCOS women have deficiencies in regulatory T-cells.

Hypothalamic Pro-Opiomelanocortin (POMC) Neuron Specific Leptin And Insulin Receptor Ko

Hill and her colleagues were interested in insulin resistance and the development of type II diabetes when they developed their Pomc-Cre, Leprflox/floxIRflox/floxmice, effectively removing both leptin and insulin receptors specifically from the POMC. [59] However, together with the anticipated glucose intolerance and insulin deficiency these mice suffer from hyperandrogenemia and polycystic ovaries. These two pathological conditions secure its eligibility as a model for the study of human PCOS.

Aromatase Inhibitors

Polycystic ovaries can be induced by androgen exposure including not only exogenous androgens but also as a result of secondary endogenous androgen excess. [60,61].The latter includes the rat PCOS model induced by letrozole, anonsteroidal aromatase inhibitor, which blocks the conversion of androgens to estrogen.[62]

HERBAL REMEDIES

1. Cinnamon

Researchers from Columbia University have found that cinnamon supplementation can help improve menstrual cyclicity in women with PCOS. Plus, a pilot study published in the journal Fertility and Sterility indicates that this herb can help reduce insulin resistance in women with PCOS.

2. Flaxseed

Flaxseed can also be used to combat PCOS as it helps decrease androgen levels. It contains lignans that increase the production of sex hormone binding globulin (SHBG) that binds testosterone in the blood, thereby preventing it from wreaking havoc in the body. Plus, being high in fiber, flaxseed helps slow down glucose metabolism and lower cholesterol levels. The omega-3 fatty acids in this superfood also reduce inflammation, lower blood pressure and reduce the risk of chronic diseases like heart disease.

3. Spearmint Tea

Spearmint tea can also help deal with PCOS due to its anti-androgenic properties. Spearmint tea can help reduce hirsutism, or excess body hair, by reducing free and total testosterone levels and increasing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels.

4. Apple Cider Vinegar

Apple cider vinegar is also beneficial for dealing with PCOS because it helps control blood sugar and keeps away from producing too much insulin.

5. Fenugreek

Fenugreek promotes glucose metabolism in the body and improves insulin resistance. This in turn helps balance your hormones. It may also help lower cholesterol, aid weight loss and promote healthy heart functioning.

6. Saw Palmetto

This herb acts as an anti-androgen, blocks 5-alpha-reductase activity and reduces the conversion of the testosterone into a more active form called dihydrotestosterone (DHT). This in turn may help prevent hirsutism or excessive hairiness in women with PCOS. It also helps thinning hair grow back.

7. Naturopath often suggest this herb for the treatment of PCOS because it helps correct the hormonal imbalance.

8. Liquorice:

This herbs act the effect on androgen metabolism in nine healthy women 22–26 years old, in the luteal phase of the cycle. They were given 3.5 g of a commercial preparation of liquorice (containing 7.6% W/W of glycyrrhizic acid) daily for two cycles.[63-65]

LIFESTYLE CHANGES

Weight loss, consuming low-calorie diet, moderate exercise activities might improve the condition. For Healthy lifestyle

- A small amount of weight loss is likely to help balance the hormones and start up menstrual cycle and ovulation.
- Eat a balanced diet that includes lots of fruits, vegetables, whole grains, and low-fat dairy products.
- Get regular exercise to help you control or lose weight and feel better.
- Avoid smoking. Women who smoke have higher levels of androgens than women who don't smoke.

LITERATURE REVIEW

1. **Chinenye Jane Ugwah-Oguejiofor, *et al.,*(2014)**investigated the effects of *Ficusplatiphylla* on female wistar rats with esterodialvalerate (EV)- induced PCOS. Animal were divided into five groups.The positive control group received clomiphene citrate, the negative control group received distilled water and the other group received 100mg/kg, 200mg/kg, 400mg/kg of aqueous extract. All groups were dosed for 15 days, except the positive control group, which was dosed for five days. On 16th day animal weresacrificed.Hormonal assay and histopathological studies were conducted. The extract treated groups showed a lower LH/FSH ratio compared with other groups. The progesterone levels were increased, indicating luteal phase repair. The extract treated group shows reversal of ovarian morphology. These data validate for the treatment of infertility.[66]

2. **Nataji,*et al.,* (2006)**evaluatedhisto-chemicalstudy of estrodialvalerate induced poly cystic ovary syndrome in rat. The rats were divided into treatment and control groups. The treatment groups were injected with intra muscular injection of estrodialvalerate. Whereas control group were given with sesame oil. After 63 days of hormone administration rats were sacrificed, ovaries were collected and processed for histochemical studies. These studies include localization of carbohydrate using PAS method, saturated and unsaturated lipid using oil Red-o, Sudan Black B, Lipase and alkali phosphatase. It conclude that during follicular atresia and cystic follicle formation, histo chemical alteration were occurred in follicular structure.[67]

3. **Somayyehsadrefozalayi,*et al.,* (2014)**studied the effect of aqueous extract of *Foniculumvulgare*onin the kidney of experimental PCOS in female rats. Animal segregated into G1(Normal saline), G2(perse control), G3(Toxic with estrodialvalerate), G4 and G5 with low and high dose of AEFV. After 4 weeks of study rats were sacrificed. Kidney processed for light microscopy and bio chemical parameter of serum were measured. The mean value of BUN(Blood Urea Nitrogen) in PCOS treated with low dose of AEFV and non-treated was significantly increased compared with high dose

of AEFV. Moreover histo-pathological changes of kidney samples were comparable in PCOS rats with respect to treated groups with AEFV. Aqueous Extract of fennel seed showed beneficial effect on renal function in PCOSrats.[68]

4. ***Christian G Krueger, et al.,*** (2002) postulated that combining extracts of grape seed (GSD) and grape skin (GSK), primary sources of grape polyphenolics, individually shown to inhibit platelet aggregation, might enhance their individual antiplatelet effects. This hypothesis was examined in vitro (human platelets) and ex vivo (dog platelets) by studying the effects of the extracts on collagen-induced whole blood platelet aggregation. The results suggest that the components of GSD and GSK, when present in combination as in red wine, grape juice or in a commercial preparation containing both extracts, exhibit a greater anti platelet effect than when present individually.[69]

5. ***Tamaro S Hudson, et al.***, (2007) studied the inhibition of prostate cancer growth by Muscadine Grape Skin Extract (MSKE) and Resveratrol through distinct mechanisms. MSKE significantly inhibited tumour cell growth in all transformed prostate cancer cell lines. These results show that MSKE and resveratrol target distinct pathways to inhibit prostate cancer cell growth in this system and that the unique properties of MSKE suggest that it may be an important source for further development of chemopreventive or therapeutic agents against prostate cancer.[70]

6. ***Revilla, I, et al.,*** (1999) identified anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode array and mass spectrometric detection. The different options MS detection is investigated in order to achieve the best conditions for detection and identification of anthocyanins by LC–MS. A method for separation of these compounds that enables the main molecules in wines to be identified by direct analysis, without any previous preparation, is proposed. The anthocyanin composition of different red grape skin extracts and commercial monovarietal wines were determined by this method.[71]

7. ***Soares de Moura, R, et al.,*** (2010) evaluated the antihypertensive, vasodilator and antioxidant effects of a grape skin extract. Oral administration of GSE significantly reduced systolic, mean and diastolic arterial pressure in Wistar rats with desoxycorticosterone acetate-salt and N^G- nitro-L-arginine methyl ester (L-NAME) induced experimental hypertension. In the rat isolated mesenteric vascular bed pre-contracted with norepinephrine, bolus injections of GSE induced endothelium-dependent vasodilatation that was substantially inhibited by L-NAME. Lipid peroxidation of hepatic microsomes estimated as malondialdehyde production was concentration-dependently inhibited by GSE. The results from the experiment provide clear evidence that the extract has got antihypertensive, vasodilator and antioxidant action.[72]

8. ***Andrea Fernandes Emiliano, et al***., (2011) examined the effects of grape skin extract on metabolic disorders and oxidative stress in adult offspring of rats fed with a high-fat diet (HFD) during lactation. Adiposity, Plasma Triglyceride, Glucose Levels, Insulin Resistance and Plasma Oxidative Damage were decreased by the extract. From the results its concluded that the grape skin extract has got protective action on both oxidative stress and metablic disorders.[73]

9. ***Karla Maria Pereira Pires, et al.,*** (2011) studied the preventive effect of grape skin extract on pulmonary oxidative response in mice exposed to cigarette smoke (CS). The administration of GSE inhibited ALI and oxidative damage induced by CS. This is associated with decreased MMP-9 (Matrix Metallopeptidase-9) activity, decreased number of inflammatory cells in the bronchoalveolar lavage fluid, and reduced levels of lipid peroxidation. The study indicates that alteration of the oxidant-antioxidant balance is important in the pathogenesis of CS-induced ALI (acute lung inflammation) and suggests lung protective effects of GSE treatment in the mouse.[74]

10. ***Roberto Soares de Moura, R, et al.,*** (2012) investigated the effect of grape skin extract on hyperglycaemia and the insulin-signalling cascade in alloxan-treated mice. Glycemia values, insulin resistance and glucose transporter (GLUT-4) content was determined.

The results suggest that the grape skin extract has hypoglycaemic and antihyperglycemic effects, which are independent of an increase in insulin release but are probably dependent on an increase in insulin sensitivity resulting from an activation of the insulin-signalling cascade in skeletal muscle.[75]

11. ***Chantal Barthomeuf, et al.,*** (2005) noted the inhibition of sphingosine-1 phosphate- and vascular endothelial growth factor-induced endothelial cell chemotaxis by red grape skin polyphenols correlates with a decrease in early platelet-activating factor synthesis. The red grape skin polyphenolic extract prevents and inhibits angiogenesis decreases the basal motility of endothelial and cancer cells, and reverses the chemotactic effect of S1P (Sphingosine 1-Phosphate) and VEGF (Vascular Endothelial Growth Factor) on bovine aortic endothelial cells (BAECs) as well as the chemotactic effect of conditioned medium on human HT-1080 fibrosarcoma, human U-87 glioblastoma, and human DAOY medulloblastoma cells. The dual inhibition of S1P- and VEGF-mediated migration of endothelial cell and of serum-stimulated migration of U-87 cells suggests a usefulness of SGE against highly invasive human glioblastoma.[76]

12. ***Katherina Fernández, et al.,*** (2010) evaluated the inhibitory effect of grape seed and skin proanthocyanidins extracted from Vitisvinífera L on the angiotensin-converting enzyme. ACE activity was measured by quantitative HPLC, measuring the hyppuric acid (HA) produced from the hydrolysis of hippuryl-L-histidyl-L-leucine (HHL) by ACE. From the result its concluded that the grape skin proanthocyanidins has got more inhibitory power than the grape seed proanthocyanidins.[77]

13. ***HeeKyoung Jung, et al.,*** (2011) studied the inhibitory action of grape skin extract on adipogenesis- and lipogenesis-related gene expression in 3T3-L1 adipocytes through the peroxisome proliferator-activated receptor-γ signaling pathway. GSE extract treatment decreased expression of aP2, Fas, and Tnfa, known markers of adipogenesis. These findings demonstrate the antiadipogenic effects of GSE on 3T3-L1 adipocytes at the genetic level, primarily on the PPARγ signaling pathway.[78]

14. ***Shao Jian Zheng,Kequan Zhou, et al***., (2010) investigated whether Grape skin extract inhibits mammalian intestinal α-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice. This study showed that the grape skin extract (GSE) significantly inhibited mammalian intestinal α-glucosidases and reduced postprandial blood glucose by 30.9% in the streptozocin- treated male mice following starch challenge and thus can be used as a novel dietary opportunity for diabetes management.[79]

15. ***Alaa El-Din A. Bekhit, et al.,*** (2011) evaluated the in vitro Antioxidant activities, sensory and anti-influenza activity of grape skin tea infusion. The antioxidant activities (DPPH_ scavenging capacity and superoxide anion radical scavenging capacity), total phenolics, the polyphenolics profile and objective colour measurements (CIELab) were determined on freeze-dried water extracts of all five tea infusions, hibiscus and green tea. From the results its confirmed that the infusion exhibited weak antioxidant and anti viral action.[80]

16. ***Sonia Hamlaoui, et al.,*** (2012) have evalauted the protective effect of grape seed and skin extract on high dosage garlic-induced renal oxidative stress. Rats were intraperitoneally injected with garlic (5 g/kg bw) or GSSE (500 mg/kg bw) or a combination of garlic and GSSE at the same doses daily for one month. They concluded that high garlic dose induced a pro-oxidative state characterized by the Fenton reaction between H_2O_2 and free iron, inducing Ca2+ depletion, while GSSE exerted antioxidant properties and Ca2+ repletion.[81]

FOCUS OF THE PRESENT STUDY

The present study was undertaken to find out the potential activity of *Vitisviniferalinn* against Estrodialvalerateinduced Poly cystic ovary syndrome in rats.From time immemorial mankind's efforts and ultimate aim have been to seek eternal happiness. And his endeavour has been to overcome and seek appropriate remedies for things that stand in his way. Plants have played a weighty role in maintaining human health and improving the quality of human life for thousands of years and have several precious components of medicines,seasonings,beverages,cosmeticsand dyes.Herbal medicines are based on the premise that plants contain natural substance that can promote health and alleviate illness.In recent times focus on plant research has increased all over the world and large evidence has collected to show immense potential of medicinal plants used in various traditional systems.

Today we are witnessing a great deal of public interest in the use of herbal remedies.Many western drugs had their origin in plant extract.There are many herbs,which are preponderantly used to treat cardiovascular problems,liver disorder,central nervous system,digestive system,metabolic disorders and for Neuroprotective effects.Given their potential to generate significant therapeutic effect,they can be useful as drug or supplement in the treatment in the management of many diseases.Herbal drug or medicinal plants,their extract and isolated compounds have demonstrated spectrum of biological activities.Such have been used and continued to be used as medicine in folk-fore or food supplement for various disorders.

Vitisviniferacontain several medicinally active compounds including polyphenols(stilbenes and flavanoids), tannins and anthocyanins. Among the plant part the grape skin has got the highest concentration of polyphenols. The different parts of the plant Vitisvinifera Linn. has been used in different system of traditional medication for the treatment of diseases and ailments of human beings.

Vitisviniferaon Estrodialvalerate induced PCOSand also planned to study the changes. Evaluation of stage of Estrous cyclicity by vaginal smear.Evaluation of Vaginal Cytology.Evaluation of serum Hormonal parameter:

- Follicle stimulating hormone
- Luteinizing hormone
- Estradiol
- Progesterone
- Testosterone

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PLAN OF WORK

- Induction of Polycysticovary in rats using intramuscular injection of Estradiol valerate.

- Evaluation of stage of Estrous cyclicity by vaginal smear.

- Evaluation of Vaginal Cytology.

- Evaluation of serum Hormonalparameter :
  - Follicle stimulating hormone
  - Luteinizing hormone
  - Estradiol
  - Progesterone
  - Testosterone

- Determination of ovarian weight, ovarian morphology, Follicular diameter & follicular thickness.
- Histological examination of the ovaries.

PLANT PROFILE

Vitisvinifera Linn.

Family: - Vitaceae

Botanical Name	:	VitisviniferaLinn.
Family	:	Vitaceae

SCIENTIFIC CLASSIFICATION[81]

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Vitales
Family	:	Vitaceae
Genus	:	Vitis
Species	:	*V.vivnifera*

VERNACULAR NAMES[82]

English	:	Common Grapes
Tamil	:	Drakshai, Kodimundiri, Gostanidraksha, Kotumuntiri
Hindi	:	Munakka, Angur, Dakh, Drakh
Malayalam	:	Munthringya, Muntiri.
Telugu	:	Drakshakottai, Drakshai
Kannada	:	Draksha, Angura, Drakshi
Marathi	:	Draksha, Angur
Gujarathi	:	Drakh, Darakh

Bengali	:	Angurphal, Drakhyaluta, Maneka
Oriya	:	Drakya, Gostoni, Onguro
Punjabi	:	Munaca
Sanskrit	:	Gostani, Mrdvika
Urdu	:	Munaqqa
Assamese	:	Dakh, Munaqqa

DISTRIBUTION

It is cultivated in Jammu-Kashmir, Himachal Pradesh, Uttar Pradesh, Rajasthan, Punjab, Haryana, Delhi, Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu.

BOTANICAL DESCRIPTION

A large decideous tendril climber, tendrils leaf opposed often bifid. Leaves are simple,more or less deeply 3-5 lobbed, orbicular-cordate, irregularly toothed glabrous above, tomentose beneath. Flowers are long peduncled, leaf- opposed cymes, greenish or white. Fruits (berry) are globose, ovoid or oblong, varying in size, pale green or purple with 2-4 seeds which are oblong-obovoid, brown, with a discoidal tubercle on the back.

PARTS USED

Ripe fruit (dried), leaf, stem, flower, dried skin, seed

CHEMICAL CONSTITUENTS[83]

The major constituents present in grape are simple phenols, flavonoids, anthocyanins and stilbenes. The major constituentspresent in grape are simple phenols, flavonoids, anthocyanins, stilbenes.Phenols are the third most-abundant constituent in grapes; carbohydrates and fruit acids are the most- and second most-abundant, respectively. The total extractable phenolics in grapes are present at ≤10% in the pulp, 60-70% in the seeds, and 28-35% in the skin. Various phytoconstituents

have been isolated from the various parts of Vitisvinfera, mainly from shoot, leaves, fruit, seed, skin, and flower which are shown below in the table no: 1.

SL.No	PLANT PART	CHEMICAL CONSTITUENTS
1	SHOOT	• Fatty Acids: Palmitic, Stearic, Oleic, Linoleic and Linolenic Acids
2	LEAF	• Flavanols: Isomer of Quercetin and Rutin. • Terpenes: Linalool, Geraniol, and α-Terpineol • Tannins: Isovitaligin, Vitaligin, Brevilagin, Ellagitannins • Minerals: Potassium and Calcium Bitartrate, Calcium Malate • Phenolic Acids: Caffeic Acid • Essential Oil: Linalool, Geraniol, Elemol Acetate, α-Terpineol • Sugar
3	FRUIT	• Sugars: Glucose, Fructose, Galactose, Mannose, Arabinose, Rhamnose • Amino Acids :Alanine,Arginine And Proline • Tannins • Terpene : Oleanolic Acid • Anthocyanins: Malvidin, Cyanidin, Delphidin • Flavonoids: Procyanidin • Carotene,Minerals,Proteins,Waxes,Xanthophylls,Vitamins • Pectin • Fruit Acids: Malic Acid, Tannic Acid, Dehydroascorbic Acid • Sterol: Cholesterol, Sitosterol, Ergosterol,
4	SEED	• Flavanols: Catechin, Epicatechin, Taxifolin, Quercetin, Gallocatechin, Procyanidin • Semi drying oil contain palmitic, stearic, oleic and linoleic acids along with sitosterol and tocopherols
5	SKIN	• Anthocyanins: Malvidin, Peonidin, Petunidin, Delphinidin • Stilbene: Resveratrol • Flavonoids:Procyanidin, Catechin, Epicatechin • Tannins • Sugars and Minerals • Acids : Tartaric Acid
6	FLOWER	• Sesquiterpene: Valencene, α- and β-Farnesene, β-Bisabolene, α-Humulene, β- and δ-Selinene, γ-Cadinene and Nerolidol

TABLE No.1

MEDICINAL PROPERTIES[84]

Fruits

The fruits are sweet, refrigerant, laxative, demulcent, cardiotonic, haematinic, haemostatic, diuretic, aphrodisiac, rejuvenating, nervine tonic, febrifuge,depurative, antispasmodic, digestive, stomachic,expectorant and tonic. They are useful in burning sensation, dipsia, constipation, amentia, cardiac debility, haemoptysis, haemorrhages, anaemia, strangury, fever, leprosy, skin diseases, dyspepsia, colic, flatulence, cough, asthma, bronchitis, Bright's disease, goutandjaundice.

Leaves

The leaves are astringent, anodyne, diuretic, depurative and useful in cephalalgia, strangury, scabies, skin diseases, syphilis, haemorrhoids, diarrhoea, splenomegaly and vomiting.

Stem

The ash of the stem is good for arthralgia, vesical calculi, haemorrhoids and orchitis. Sap of young branches is used in skin diseases.

Flowers

The flowers are expectorant, emmenagogue and haematinic, and are useful in bronchitis, liver disorders, anaemia, amenorrhoea and dysmenorrhoea.

Skin

The grape fruit skin has got antiplatelet, anticancer and anti oxidative action. The skin extract is also used for hypertension and diabetesIt has also got anti adipogenic action**.**

PREPARATION OF EXTRACT

Hydroalcoholic extracts of Grape Skin Extracts (GSE) (yield = 7.5%) were prepared by routine methods using rotary vacuum evaporator (Roteva, Equitron, Medica Instruments Mfg.co, Mumbai) and programmable freeze dryer (Allied Frost,Mumbai) from dried Grape skin. A voucher specimen of Grape skin has been deposited at the herbarium, in the Department of Pharmacognosy, K.M.Collegeof Pharmacy (No. GS2013-09:KMCP). Powders of GS extracts are light brown color. GS extracts were stored in a refrigerator at −20◦C to protect from light and degeneration, and they are well soluble upto 60mg/mL concentration levels in distilled water used as vehicle as clear light brown solution.

Extraction Procedure

The fresh plant fruit of *Vitisvinifera*were collected and authenticated. The fruits were peeled off and the skin was dried in the shade. Then the shade – dried grape skin were pulverized to get coarse powder, sieved under mesh no.60.

Materials

- Rotary Evaporator
- 70%ethanol
- distilled water
- Shade dried grape skin powder

Methods

500gm of coarsely powdered dried skin of *Vitisvinifera* were extracted with 2 liters of 70% Ethanol at 70^{o}C temperature, for 1 hour in a 20 liter round bottom flask with condenser attached. Filter and collect the extract. Repeat extraction with 2 liters of 70% Ethanol. Filter and collect the extract. Extract the marc with2 liters of water. Filter and combine the extracts. The combined extract was evaporated to dryness under reduced pressure in a Buchi RotaryEvaporator (Switzerland) at 65°C, to obtain a brownish colour residue. This extract was used for the experimentation.

PHARMACOLOGICAL EVALUATION

To a large world population, medicinal plants are the only source to prevent and treat various diseases.Medicinal plants form main source of health care due to better acceptability and fewer side effects. Herbal plants have been used since centuries to correct disorders caused by the hormonal imbalance related to female reproductive system.[91-93] Current research work is to investigate the effect of HAEGSE in treatment of Estradiol valerate induced PCOS. The current research work focuses on normalization of estrous cycle in PCOS after treatment with *Vitisvinifera*Linn.

EXPERIMENTAL MODEL

For the study of poly cystic ovary syndrome an experimental model is selected in such a way that it would satisfy the following condition

- The animal should develop cyst rapidly.
- Pathological changes in the site of induction should result from PCOS formation.
- The symptom should be ameliorated or prevented by a drug treatment effective in humanbeings.
- The drug tested should be administered orally.
- Drug dosage should approximate the optimum therapeutic range for human, scaled the test animal weight.

Selection grouping and Acclimatization of Laboratory Animal

Female albino rats (180-220gm) are produced from animal experimental laboratory, and used thoughout the study. They are housed in micro nylon boxes in a control environment (temp 25+20c) and 12 hrs dark\ light cycle with standard laboratory diet and water *ad libitum*. The study is conducted after obtaining institutional animal ethical committee clearance. As per the standard practice the rats are segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They are fed on healthy and maintained in hygienic environment in our animal house.

Technique for inducing PCOS

Technique for induction of PCOS in animals by chemically induced (using Esterodialvalerateoil, Letrazole, Androgen, Prenatal Antrogen, Dehydroepiandrosteroneetc)have been used in experimental studies of PCOS activity.

Induction of PCOS in the Animals

In the present study, Estradiol valerate induced PCOS is used to evaluate the treatment of PCOS.[79]Sixty adult virgin Wistar rats of approximately 10-12 weeks of age, weighing between 180-220gm and with regular 4-5 day estruscycles as assessed by vaginal smear, were used for the study [85]. Five of the rats were kept as controls, and the others were each given intramuscular injection of 4 mg EV in an oily solution per rat.[86,87] Vaginal smears were examined daily in all animals. Cessation of cyclicity, which was shown by the persistent cornification of vaginal smears, was used as a criterion forselection into the PCOS group.

Vaginal Smears

The stage of cyclicity was determined by microscopic analysis of the predominant cell type in vaginal smears. Estrous cyclicity was monitored by vaginal smears obtained between 0800 and 1200 hours, and it was assessed by analysis at the light microscopy level of the relative proportion of leukocytes, epithelial and cornifiedcells found in daily vaginal lavages, which characteristically change during different stages of the estrous cycle. The rat estrous cycle (estrus, diestrus1, diestrus2, and proestrus) usually lasts about 4 days, in controls or PCO rats**.**[88]

Vaginal cytology

Vaginal smears were obtained daily. The rats were held at the thorax, ventral side upper, whilst providing lumbar support. Vaginal secretions were collected using cotton-tipped with a drop of physiological saline. After about 1-2 inches of the swab was inserted into the vagina of the female rats and the end was rotated through 2-3 revolutions (which allowed the cotton tip to pick an adequate load of cells), the swab was then gently withdrawn and the tip of the cotton rolled along the length of a glass slide. The dried smear was fixed by dipping it in a container of 70% alcohol. The slides were then stained with 0.5% methylene blue solution, rinsed in tap water and

examined under ×10 objective lens (without the condenser lens) of a light microscope.[89,90]

Treatment Protocol

Thirty animals of Wistar rat were randomly selected and divided into five groups (n=6) and housed as such (6 rats per cage). All the animals in four groups were injected with Estradiol valerate by intra muscularly and the remaining one group is normal control group. The rats were allowed to establish PCOS for 30 days.[91] After 30 days, groups in G4 & G5 were dosed orally by gavage for 15 days, whereasrats in the standard group was dosed for 5 days.

Group 1 served as the normal control.

Group 2 served as the PCOS control. Group 1 and 2 receives normal diet and water.

Group 3 served as the positive control, was treated with injection Clomiphene citrate at 20 mg/kg body weight, Intra peritoneally.[92]

Group 4 served as the treatment control, treated with Hydroalcoholic extract ofGrape skin extract(HAEGSE) at 200 mg/kg body weight, through orally.

Group 5 served as treatment control which was treated with Hydroalcoholic extract ofGrape skin extract(HAEGSE) at 400 mg/kg of body weight, through orally.[93]

On 16th day, Six animals from each group (Control and PCO) were randomly selected and anaesthetised with ether. Blood samples were collected by retro orbital puncture, and the serum were used for hormonal assays (FSH, LH, estradiol, progesterone andtestosterone).[91] The ovaries were excised and weighed, and histopathological examination was conducted on the ovaries.

Serum hormonal assays

Serum testosterone, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and estradiol were measured usingan enzyme immunoassay kit for the quantitative determination ofthe corresponding hormones.

Organ weight:

On the 16th day, some body organs of rats in all treatment groups except the Positive group were excised and weighed; organs from rats in the Positive group were instead excised and weighed on the 6th day.

Histopathological examination:

The excised ovaries were fixed inBouin'ssolution. They were dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax that melted at 60^{o}C. Serial sections were mounted on 3-aminopropyl triethsilane-coated slides and dried for 24 hours at 37ºC. The sections on the slides were deparaffinised, hydrated and stained with MayerÊshematoxylin and eosin dyes; they were then dried and mounted for histology. The ovaries were viewed at 40x magnification using the Scope photo 3.0 imaging device (Scope Tek DCM 200 (USB 2.0, Hangzhou Scope tekOpto-Electric Co Ltd). The diameter and thickness of the cystic follicles were measured. The cystic follicles were defined by thickened and fibrotic cortex with a prominent outer theca and internal layer.[91]

Histology

The ovaries from toxic controls (EV-treated), standard &treated groups were removed, cleaned of adherent connective fat tissue, andtissue samples were fixed in 10% formaldehyde buffer for histological examinations.Ovaries were imbedded in paraffin, cut in 8-µm sections, and stained with hematoxylin and eosin examined by light microscopy [hematoxylin and eosin staining) Ovaries were examined for evidence of polycystic morphology, as described previously.[81]

Statistics

The results are expressed as Mean ± SEM. Data was evaluated using ONE WAY ANOVA followed by Newman – Keul's multiple range test. Probability values less than ($p< 0.01$) were considered significant.

RESULTS

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE) on LH in EV induced PCOS rats

Estradiol valerate (EV) treatment causes significant rise in LH levels & lowering of FSH levels in toxic control (G2) compared to normal control groups (G1) at P<0.001. The LH /FSH ratio was significantly different from the control groups. An elevated LH/FSH ratio observed in toxic group whereas extract treated groups i.e., both doses of (HAEGSE)(200mg and 400mg/kg)showed a lower LH/FSH ratio & significant decrease (p<0.01) in LH & rise in FSH levels when compared to toxic control group. (Table: 2)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE) Estradiol in EV induced PCOS rats

There was statistically significant decrease in estradiol levels with Estradiol valerate (EV) injection after 30 days (p<0.01). Concurrent administration of HAEGSE for 15 days showed significant rise in estradiol levels (p<0.01). Animals in Standard group also showed significant rise in estradiol levels**.** (Table: 2)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE)on progesterone in EV induced PCOS rats

Toxic control group i.e., treated with estradiol valerate had shown significant lowering of progesterone. But treatment with HAEGSE at both doses (200mg/kg and 400mg/kg) along with EV was able to increase the progesterone levels (p<0.001) to near normal values significantly. Similar results were also observed in standard group. (Table: 2)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE)on testosterone in EV induced PCOS rats

There was no significant rise in testosterone levels after exposure of rats to estradiol valerate ($p<0.01$) for 30 days. Treatment with HAEGSE at two doses 200mg/kg and 400mg/kg for 15days doesn't show any significant changes in testosterone levels. Similar results were observed after clomiphene treatment. (Table:2)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE)on ovarian morphology:

Ovaries of toxic control (Estradiol valerate) group exhibited more cystic follicles compared with other groups but these were not evident in extract control group. Both the 200mg/kg & 400mg/kg showed normal follicle at different stage of development. There was evident of arteric follicles present in 200mg/kg. The group that received 400 mg/kg showed numerous healthy developing follicles. (Table: 3)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE) on follicular diameter & thickness:

The follicular diameter & thickness of the cysts in PCOS treated group were increased whereas it was reduced in standard & extract treated groups. (Table: 3)

Effect of Hydroalcoholic extract of grape skin extract (HAEGSE) on ovarian weight:

The ovarian weight of EV control group showed a significant decrease ($p<0.01$), when compared with other groups, whereas in treatment control group 200mg/kg & 400mg/kg it was restored to near normal values. (Table: 3)

Table No.2 Effect of Grape skin extract on serum hormone in Estradiol valerate induced PCOS

GROUP	LH	FSH	Estradiol	TSN	PRGSN
G1	6.146±0.33	8.27±0.25	54.12±2.31	0.28±0.04	14.15±0.61
G2	11.36±0.75**a	2.26±0.235**a	14.32±0.82**a	0.37±0.02**a	7.055±0.712**a
G3	5.25±0.38	7.02±0.46	46.49±1.74	0.32±0.02	12.12±0.29
G4	3.70±0.18**b	6.63±0.53**b	38.17±1.33**b	0.34±0.01**b	10.8±0.72*b
G5	4.57±0.21**b	6.13±0.56**b	41.18±0.92**b	0.32±0.01**b	11.60±0.76*b

G_1- Normal, G_2-Toxic, G_3-Standard,

G_4-Low dose (HAEGSE), G_5-High dose(HAEGSE)

All values expressed as means ± SEM for 6 animals in each group.

**a- Values are significantly different from Normalcontrol (G_1) at P<0.001

**b- Values are significantly different from PCOS control (G_2) at P<0.001

*b- Values are significantly different from PCOS control (G_2) at P<0.01

Table No.3 Effect of Grape skin extracts on ovarian morphology of PCOS rats

Dose mg.kg ovarian feature	Normal	Toxic control	Std control	Low dose	High dose
Atretic follicle	0.00±0.00	4.43±0.307	1.12±0.05	3.06±0.17**b	0.05±0.11*b
Cystic follicle	0.00±0.00	10.55±1.21	3.7±10.58	0.00±0.00	0.00±0.00
Cystic follicle diameter	0.00±0.00	87.73±2.367	71.19±2.35	0.00±0.00	0.00±0.00
Cystic follicle thickness	0.00±0.00	42.35±1.48	34.63±2.18	0.00±0.00	0.00±0.00

G_1- Normal, G_2-Toxic, G_3-Standard,

G_4-Low dose (HAEGSE), G_5-High dose (HAEGSE)

All values expressed as means ± SEM for 6 animals in each group.

**b- Values are significantly different from PCOS control(G_2) at P<0.001

*b- Values are significantly different from PCOS control(G_2) at P<0.01

CHART.1 - LUTEINISING HORMONE

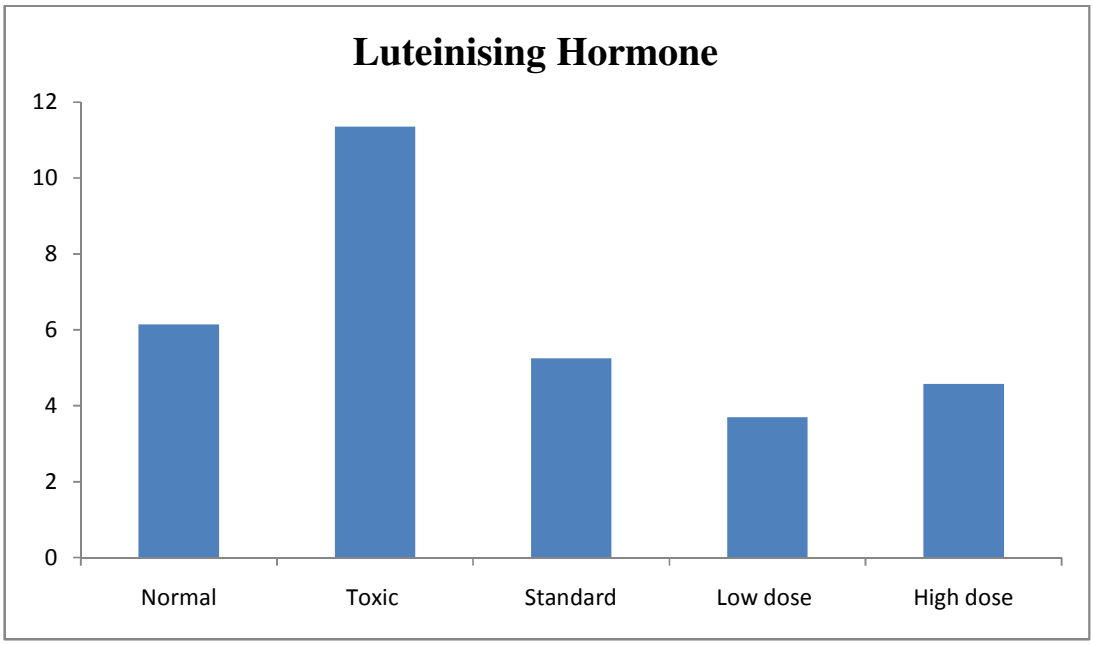


CHART.2 - FOLICLE STIMULATING HORMONE

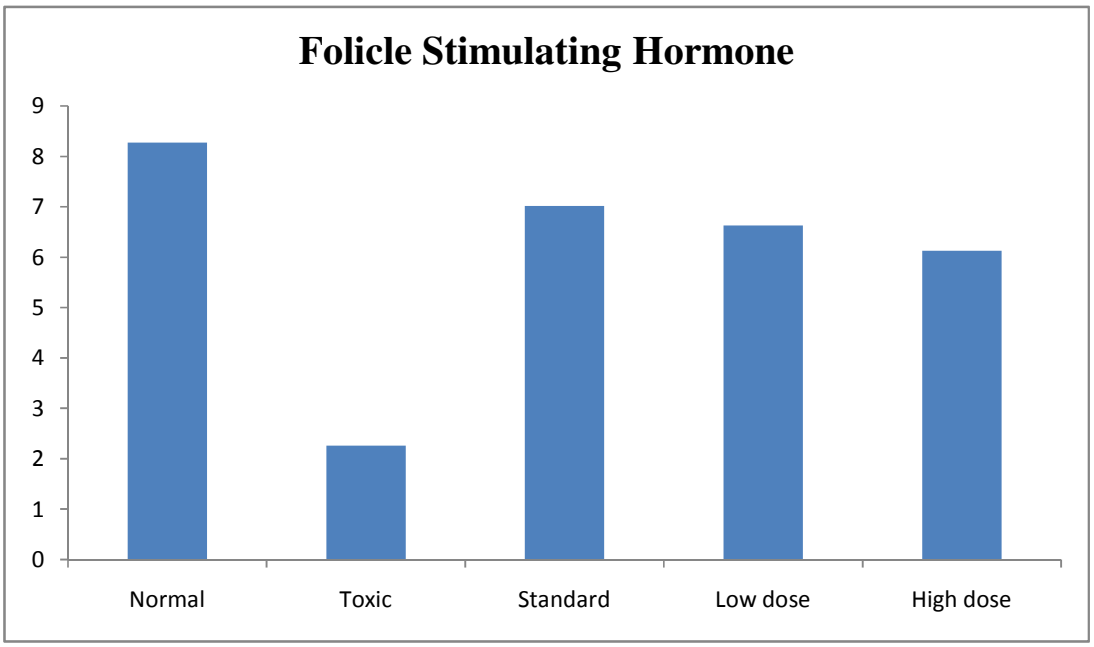


CHART.3 - ESTRADIOL

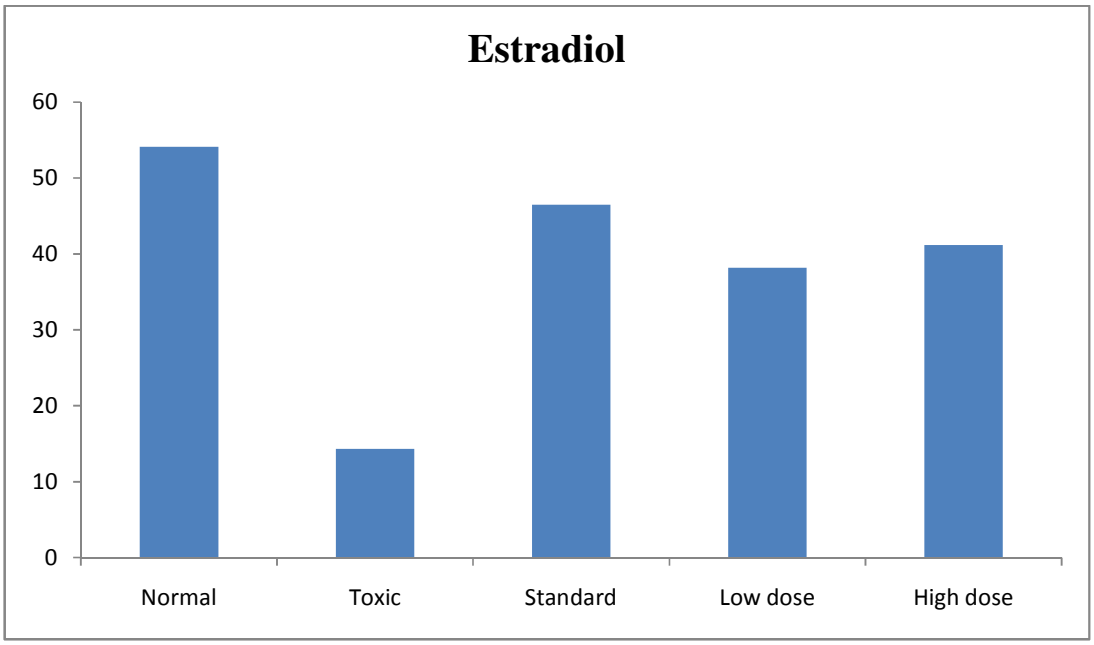


CHART.4 - TESTOSTERONE

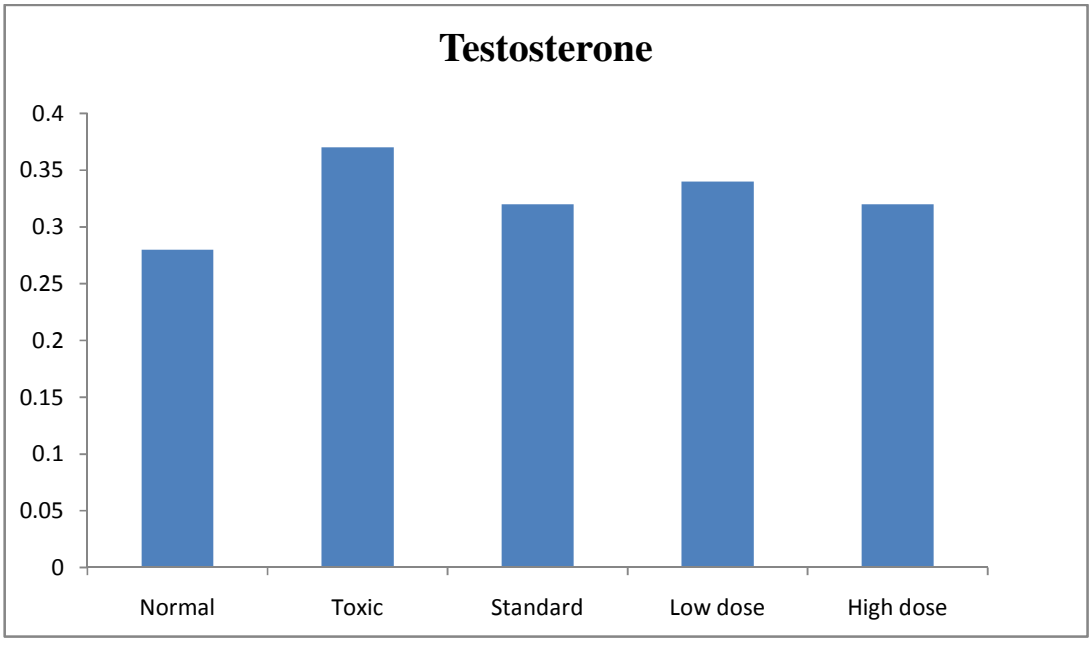
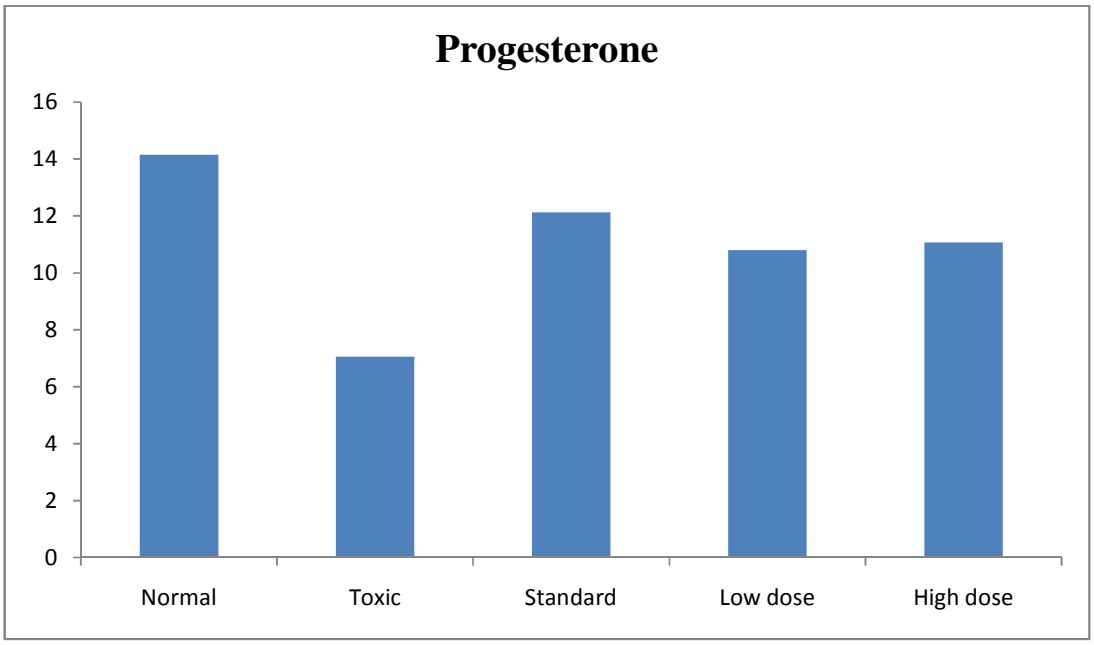


CHART.5 - PROGESTERONE



HISTOPATHOLOGICAL EXAMINATION

G1: NORMAL CONTROL (10ml/kg Normal saline)

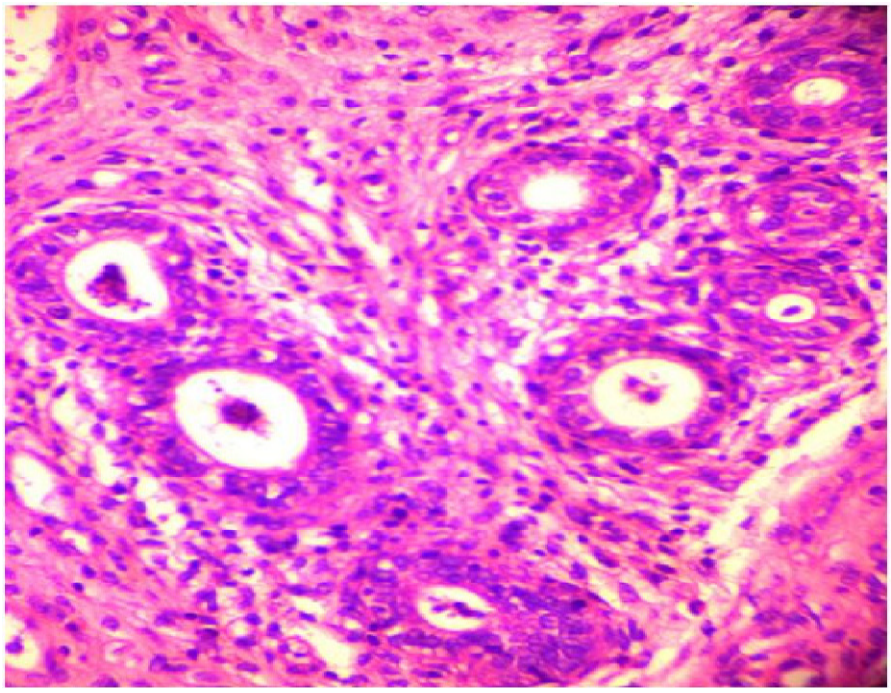


Fig.13

Section of ovary from normal rat showing the presence of antralfollicle,Carpusluteum, oocyte surrounded by Granulosa cells and theca layer

G2: TOXIC CONTROL (Estradiol valerate4mg/kg)

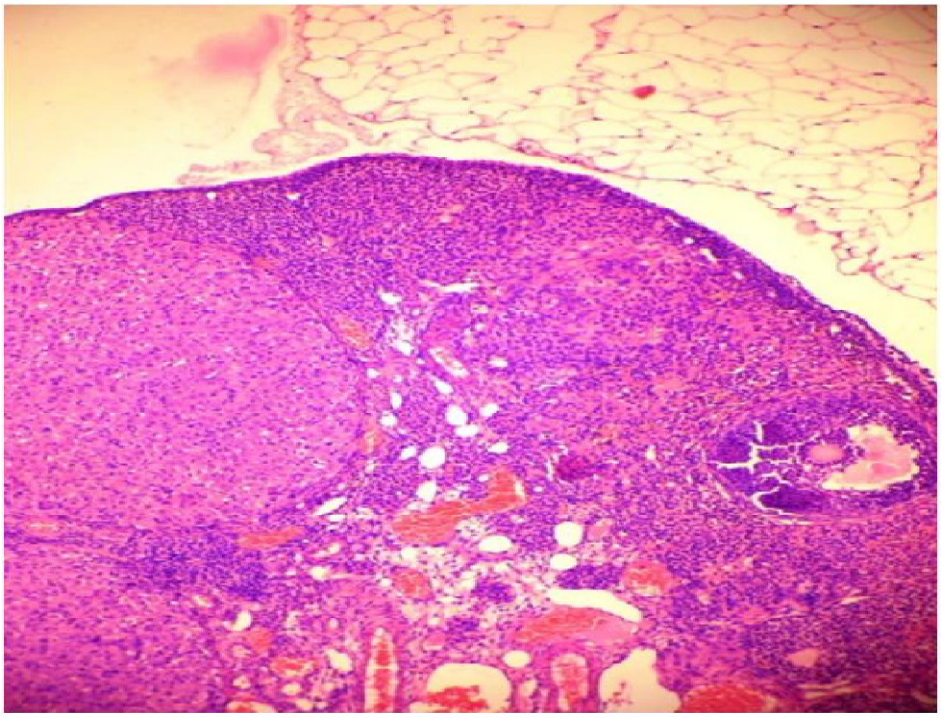


Fig.14

Section of ovary from PCOS rat exhibiting many cystic degenerating follicle and arteric follicle with degenerated granulosa layer

G3: STANDARD CONTROL (Clomiphene Citrate 20mg/ml)

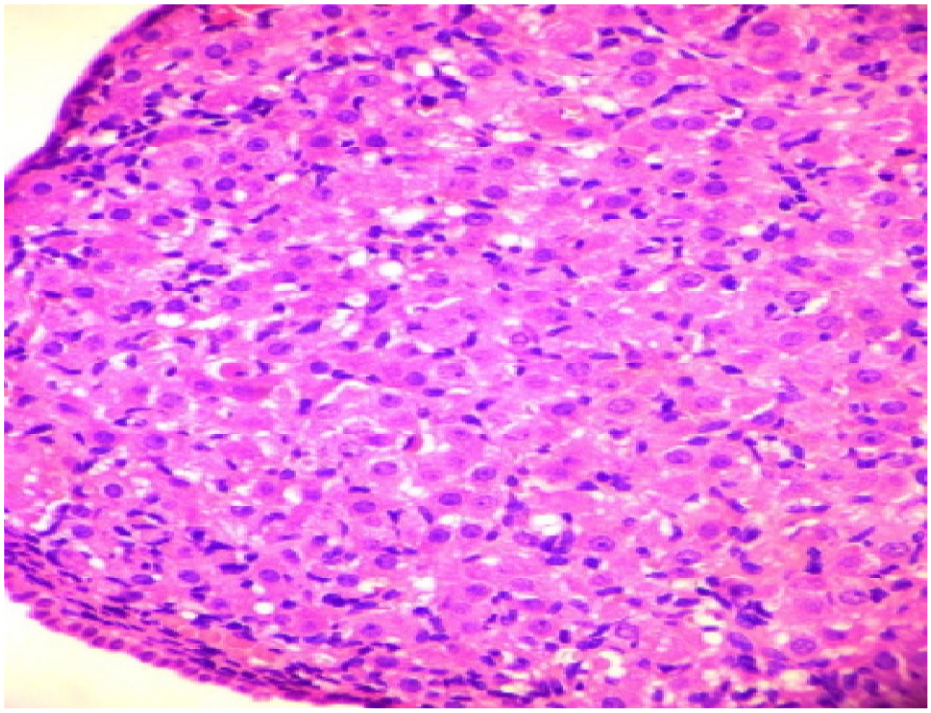


Fig.15

Section of ovary from PCOS rat treated with clomiphene citrate showing the presence of normal follicle with clearantrum, Oocyte in granulosa layer.

G4: TREATMENT CONTROL

(LOW DOSE HYDROALCOHOLIC EXTRACT OF GRAPE SKIN EXTRACT (HAEGSE) 200mg/kg)

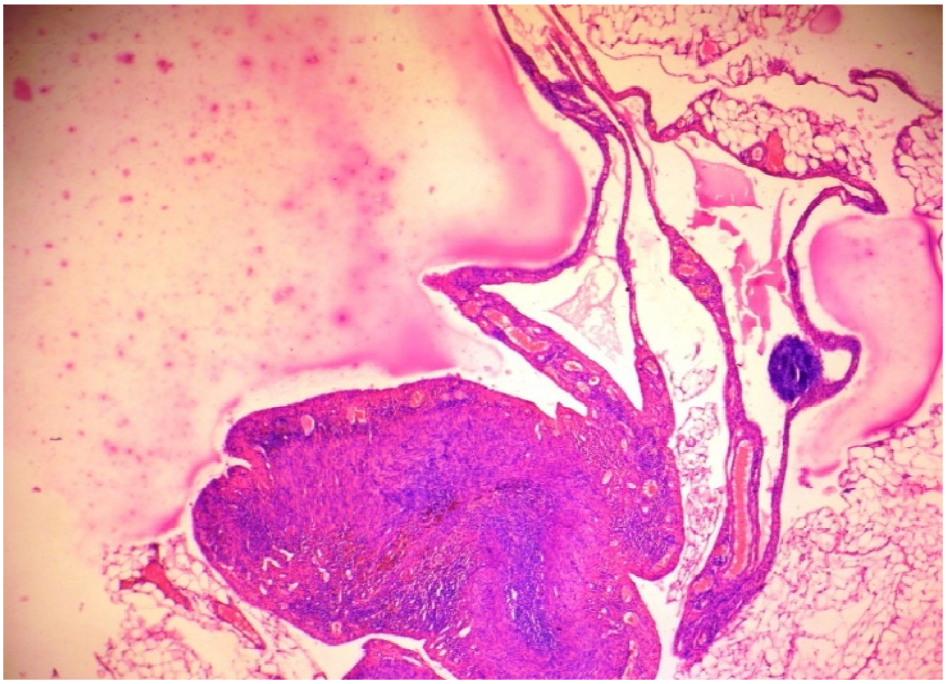


Fig.16

Section of ovary from PCOS rat treated with low dose of Hydroalcoholic Extract of grape skin extract 200mg/kg shows mild degenerative follicle and absence of cystic, arteric follicle

G5:TREATMENT CONTROL

(HIGH DOSE HYDROALCOHOLIC EXTRACT OF GRAPE SKIN EXTRACT (HAEGSE) (400mg/kg)

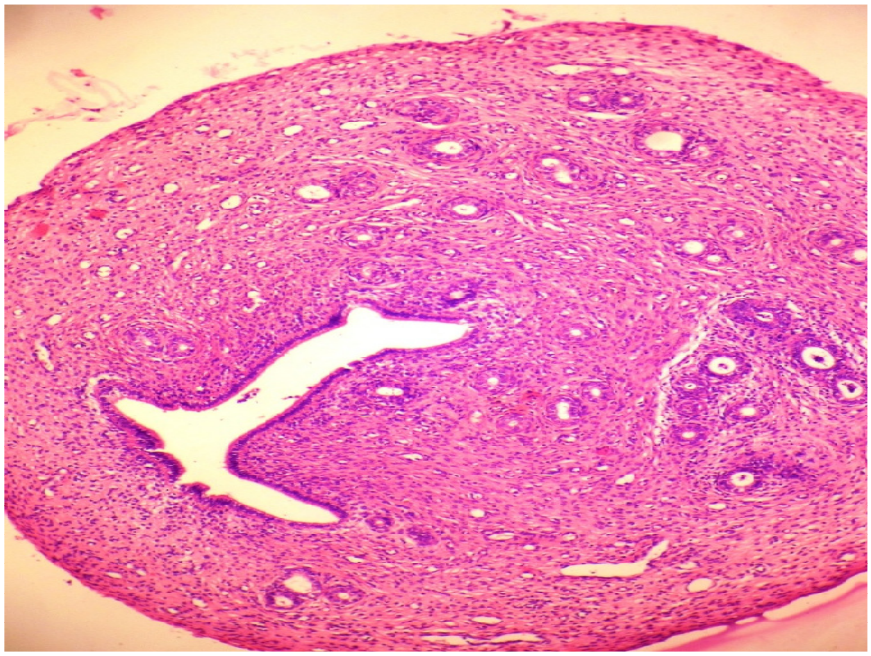


Fig.17

Section of ovary from PCOS rat treated with high dose of Hydro alcoholic Extract of grape skin extract 400mg/kg shows the presence of developing regenerating follicle and carpus luteum,Oocytes with in Granulosa layer

DISCUSSION

PCOS has been considered a progressive multiglandularendocrinopathy where thedelicatebalance of the hypothalamic–pituitary–adrenal– ovarian axis is disturbed, resulting in a failure of thecyclic reproductive mechanism.[94,95]A total loss of the cyclicreproductive changes appears to follow a phase of irregular rhythmicity in rats experimental PCOS.[96]The precise cause of polycystic ovarian syndrome is unknown; however, it is considered to be a complex multi-genetic disorder characterized by disordered gonadotropin release, dysregulation of steroid genesis, insulin insensitivity, chronic annovulation, menstrual irregularities, clinical or biochemical hyperandrogenism, and ultrasound data of polycystic ovaries.[97,98,99]

Although many models can be used to study PCOS, induction of PCOS by Estradiol valerate can also be considered as one of the best model for studying PCOS. Hence, in terms of exhibiting the majority of reproductive and endocrine symptoms associated with PCOS, rodent PCOS models appear to closely parallel the human condition.This study investigated the effect of Hydroalcoholic extract of *Grape skin extract* on the serum levels of LH, FSH,estradiol, testosterone & progesterone in EV induced PCOS. After 30 days of PCO induction, animals were analysed both harmonically &histologically, on 16th day after treatment with hydroalcoholic extract, animals were also analysed irrespective of their estous cycle.

In PCOS condition, normal gonadotropin-ovarian axis is disturbed results in hormonal imbalancereflected by the higher levels of LH, lower FSH levels and reversal of LH: FSH ratio. An elevated LH/FSH ratio and anovulation are typical findings in women with PCOS.[100,101]The mechanism for this LH hyper secretion is not entirely clear, but recent data suggest that in anovulatory PCOS condition, the predominant reason for high serum LH concentrations is abnormal negative feedback on LH secretion mediated by either estradiol or progesterone.[102]The extract treated groups shows better reduction in this LH/ FSH ratio indicate that extract could reverse PCOS condition.

Oestrogen similar to other steroids become altered in PCO.[103]Normally, testosterone and androstenedione are converted to estradiol and estrone, respectively, with the help ofcytochrome P450 aromatase, which plays an important role in ovary's hormonal balance. The level of estradiol is very minimum in PCOS rats since the metabolic conversion is very slow.Repetitive administration of HAEGSE led to significant rise in estradiol.Similarly, the reduction in the level of progesterone in the PCOS-induced animals could be responsible for the persistentoestrusphase.[104]Elevation in the concentration of serum progesterone by HAEGSE may be responsible for the reversal of the luteal phase dysfunctionand restoration of normalcy of the estrous cycle. Our study showed that Grape skin extractinduced an increase in serum estradiol implies that plant causes marked improvement in endocrine function and recovery of ovulary functions in the rats.Hyperandrogenism (as a result of high testosterone levels) which is evident in human PCOS.[105,106] was not present in this animal model of EV induced PCOS.[107] Therefore no effect of the extract on androgen levels was observed using this model of PCOS induction.

Ovarian weight in PCOS induced rats was more than the normal rats which is in accordance with earlier findings.[108-111]Treatment with HAEGSE prevented further increase in ovarian weight & returned to normaly.The biochemical results are also supported by histopathological observation of light microscopy.Thehistomorphometry of PCO was a suitable measurement for describing the cystic status because differences were observed in the morphological characteristic and in the presence or absence of follicular cysts.[119]It is reported that the histopathological study of PCOS induced rats shows the formation of poly cysts in the ovary.[112,113]Ovaries exhibited increased follicle atresia and multiple cysts with thin granulose cell layers and thickened theca cell layers.[114]After treatment with extract of *Grape skin extract*PCO condition was reversed, number of cystic follicles reduces & foundnumerous healthy follicles at different stage of development. Thisindicate that treatment group shows marked recovery of ovarian tissue and the animals may probably be preparing for ovulation.

Furthermore treatment with HAEGSE brought back feedback inhibition of gonadotropin (LH & FSH) along with corresponding increase in estradiol &progesterone.All these together emphasize the ability of extract in attenuating clinical, biochemical, histological features of PCOS. The presence of flavonoids in HAEGSEmight account for pharmacologicaleffect.[115]Again since PCOS condition has been reported to reduce the level of antioxidant enzyme/molecule apart from that flavonoids reported to posses playing an antioxidant role in PCOS rats.[116]Antioxidants play an important role in protecting the human body against damage from reactive oxygen species.[117] Plants containing phenolic compounds, in particular flavonoids have been reported to exhibit strong antioxidant properties.[118]These phytochemicals may be responsible for acclaimed folklore use of plant in management of gynecological problem.

PLANT PROFILE

Fig No: 12

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CONCLUSION

In PCOS women, normal gonadotropin-ovarian axis is disturbed. This is reflected by the higher levels of LH, lower FSH levels and reversal of LH: FSH ratio. FSH levels in PCOS show lower than normal values whereas after treatment it proves significant variations. Androgen dynamics involving testosterone doesn't show any significant variation. Estrous cycle of the rats was disturbed when they were in polycystic ovary condition were returned to normalcy after treatments. The reproductive system and ovary weight of normal rats increased with estradiol valerate injection which normalizes after treatment with*Grape skin extract*.Polycyst observed in all groups except normal control was completely reduced. Polycystic Ovarian Syndrome (PCOS) is one of the most common female endocrine disorders which may leads to infertility. Herbal drugs have promising role in treatment of PCOS and shows steady effect with minimal side effects. Herbal drugs enhance immunity of the body also regularize menstrual cycle without fluctuating hormonal level. In conclusion Hydroalcoholic extract of *Grape skin extract* shows significant recovery of FSH,LH, estradiol & progesterone and also restored the irregular cycle and ovarian physiology to normal in the PCOS animals. Thus*Grape skin extract* was effective in reversing the hormonal imbalance induced by estradiol valerate in PCOS & validate the use in treatment of infertility.